DESIGN AND SYNTHESIS OF CHEMICAL PROBES FOR THE PLEKSTRIN HOMOLOGY DOMAIN

Thomas Elliott

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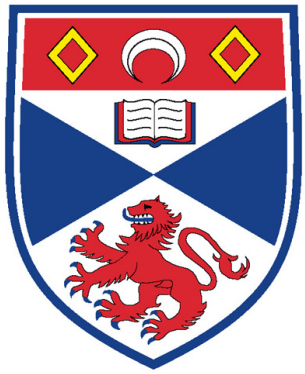
Design and synthesis of chemical probes for the plekstrin homology domain



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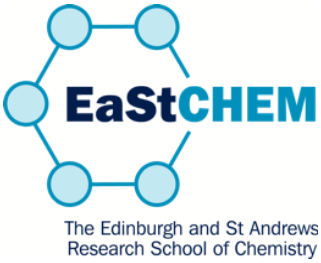

School of Chemistry and

Centre for Biomolecular Sciences

Thomas Elliott

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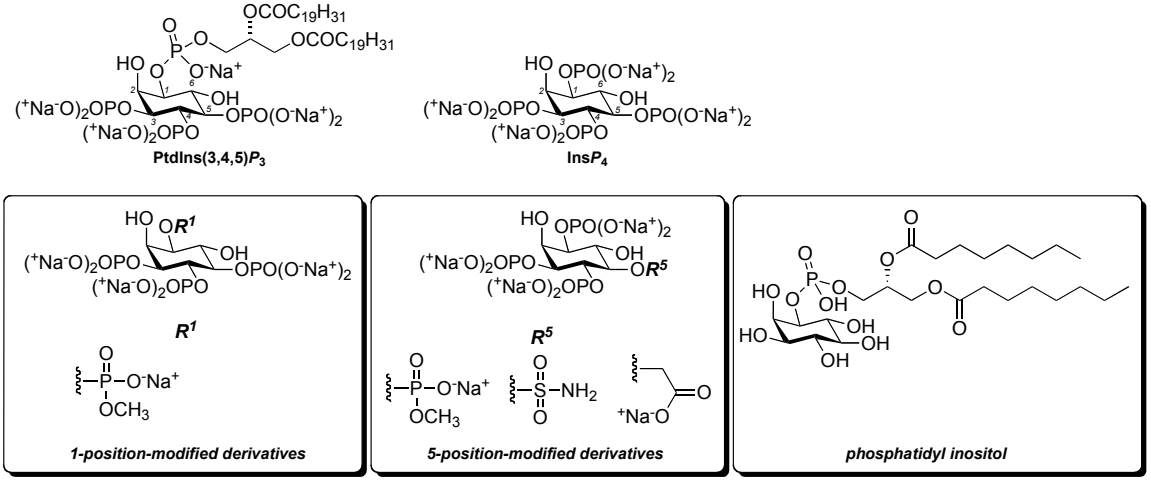
Thesis submitted to the University of St Andrews in the application for the degree of Doctor of Philosophy

Supervisor: Dr Stuart Conway

Abstract

The phosphatidylinositol polyphosphates play a fundamental role in intracellular signalling. Of particular importance is phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3], which acts by recruiting effector proteins to the cell membrane. PtdIns(3,4,5)P_3 interacts with its protein targets through selective binding domains that include the pleckstrin homology (PH) domain. The PH-domain-containing kinase, protein kinase B (PKB/Akt), which interacts with PtdIns(3,4,5)P_3, is upregulated in ~15 human malignancies. Significantly, inhibition of the PtdIns(3,4,5)P_3-PKB interaction has proved viable as a point of therapeutic intervention.



There is currently a lack of small molecule probes that selectively interact with a given PH domain. Consequently, it is impossible to dissect the cellular function of PH-domain-containing proteins at a molecular level. To address this problem, we have designed and synthesised a number of derivatives of the PtdIns(3,4,5)P_3 inositol head-group – Ins(1,3,4,5)P_4. Replacement of the 5-position phosphate with a range of phosphate bioisosteres afforded compounds that displayed no binding affinity for the PH-domain of general receptor for phosphoinositides 1 (GRP1). However, it was shown that the 5-position sulfamate analogue displayed selectivity for the PH-domain of PKB. The methylphosphate biosiostere at the 1-position displayed binding for both the GRP1 PH-domain as well as the PKB PH-domain. These results demonstrate that subtle modification of the Ins(1,3,4,5)P_4 structure allows the synthesis of compounds that interact selectively with a given PH domain. We will now use these results for the synthesis of a second generation of compounds with improved PH-domain affinity and selectivity.

Declarations

I, Thomas Elliott, hereby certify that this thesis, which is approximately 58,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in September 2006 and as a candidate for the degree of Doctor of Philosophy in October 2009; the higher study for which this is a record was carried out in the University of St Andrews between 2006 and 2010.

Date Signature of candidate

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Date Signature of supervisor

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Table of Contents

List of Abbreviations

All	allyl
AllBr	allyl bromide
ADP	adenosine diphosphate
Arg	arginine
ARNO	ARF nucleotide-binding site opener
ATP	adenosine trisphosphate
BAD	Bcl-2-associated death promoter
*Ba*PlsY1	*B. anthracis* acyltransferase
BM	butyryl oxymethyl
BnBr	benzyl bromide
Btk	Bruton's tyrosine kinase
c	concentration
CAN	ceric(III) ammonium nitrate
CoA	coenzyme A
COSY	correlation spectroscopy
CSA	camphor sulfonic acid
CSI	chlorosulfonyl isocyanate
DAG	diacylglycerol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	dichlorodicyanoquinone
DGD	*N*-acetyl-D-glutamate
DIBAL-H	diisobutylaluminium hydride
DMA	dimethyl acetamide
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ENTH	epsin *N*-terminal homology
FKHR	forkhead (Drosophila) homolog 1 (rhabdomyosarcoma)

FRET	fluorescence resonance energy transfer
Glu	glutamate
GSK3β	glycogen synthase kinase 3-beta
GRP1	general receptor for phosphoinositides-1
HM	hydrophobic motif
HMBC	heteronuclear multiple bond coherence
HOBT	hydroxybenzotriazole
HSQC	hetronuclear single quantum coherence
IC50	inhibitory concentration 50%
Ins(1,4,5)P_3	1D-*myo*-inositol (1,4,5)-trisphosphate
Ins*P*4	1D-*myo*-inositol (1,3,4,5)-tetrakisphosphate
Ins*P*6	1D-*myo*-inositol (1,2,3,4,5,6)-hexakisphosphate
Ins*P*7	1D-*myo*-inositol (1,2,3,4,5,6)-heptakisphosphate
Ins*P*8	1D-*myo*-inositol (1,2,3,4,5,6)-octakisphosphate
IR	infra red spectroscopy
KD	kinase domain
*K*i	inhibition constant
LPA	lysophosphatidic acid
lys	lysine
*m*CPBA	3-chloroperoxybenzoic acid
MDM2	protein coded for by Mdm2 gene
MOM	methoxymethyl ether
mTOR	mammalian target of rapamycin
NagA	*N*-acetyl-D-glucosamine-6-phosphate
NF-κB	nuclear factor – kappa B
NMR	nuclear magnetic resonance
Np_nN	dinucleoside polyphosphate
NSCLC	non-small cell lung carcinoma
p53	protein 53
PDK 1/2	phosphoinositide dependent kinase 1 or 2
Pg	protecting group

PH	pleckstrin homology
Phe	phenylalanine
PI	phosphoinositide
PI3K(I)	type I phosphoinositide 3-kinase
PI3K(III)	type III phosphoinositide 3-kinase
PI4K	phosphoinositide 4-kinase
PI5K(I)	type I phosphoinositide 5-kinase
PKBα	protein kinase B α isoform
PKB PH	protein kinase B pleckstrin homology
PKC	protein kinase C
PLC	phospholipase C
PMB	4-methoxybenzyl
PMBCl	4-methoxybenzyl chloride
PPM	parts per million
PTB	phosphotyrosine-binding domain
PtdIns	phosphatidylinositol
PtdIns(3)*P*	phosphatidylinositol (3)-phosphate
PtdIns(3,4)P_2	phosphatidylinositol (3,4)-bisphosphate
PtdIns(3,4,5)P_3	phosphatidylinositol (3,4,5)-trisphosphate
PtdIns(4)*P*	phosphatidylinositol (4)-phosphate
PtdIns(4,5)P_2	phosphatidylinositol (4,5)-bisphosphate
PtdIns(5)P	phosphatidylinositol (5)-phosphate
PTEN	phosphatase and tensin homologue
PTP	protein tyrosine phosphatase
PTSA	4-toluenesulfonic acid
pTyr	phosphotyrosine
PX	phox homology
rac	racemic
RNA	ribonucleic acid
RT	room temperature
SAR	structure activity relationship

Ser	serine
SGK	serum/glucocorticoid regulated kinase
SH2	Src homology domain
SHIP 1/2	Src homology 2-containing inositol 5'-phosphatase 1 or 2
*Sp*PlsY	*S. pneumoniae* acyltransferase
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TCA	trichloroacetimidate
TES	triethylsilyl
TFA	trifluoroacetimidate
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropylsilyl
TIPSOTf	triisopropylsilyl trifluoromethanesulfonate
TLC	thin layer chromatography
TMS	tetramethyl silane
4-TsOH·H_2O	4-toluenesulfonic acid monohydrate
UDP	Uridine diphosphate
VL	variable loop
XLA	X-linked agammaglobulinemia

1. Introduction Part 1

1.1. Inositol Structure and Nomenclature

Small natural products and bio-molecules are often of great interest to scientists due to their discrete functions in biochemical systems and their specific interactions with biological macromolecules. Their small size makes them an attractive target for organic chemists, because of the potential for making synthetic analogues, which can, in turn, provide useful tools for probing and investigating biochemical systems.

One such natural product of interest is *myo*-inositol **1**, which is one of 9 naturally occurring and unnatural inositol isomers (Fig 1.1). Inositol consists of a six membered carbocyclic ring that bears a single hydroxyl group at each carbon of the ring. The stereochemistry possessed by each hydroxyl group around the ring dictates the isomer of inositol. *myo*-Inositol **1** is the most abundant of the five naturally occurring inositols (*myo*-inositol **1**, *neo*-inositol **3**, D-(+)-*chiro*-inositol **8**, L-(-)-*chiro*-inositol **9**, and *scyllo*-inositol **2**).

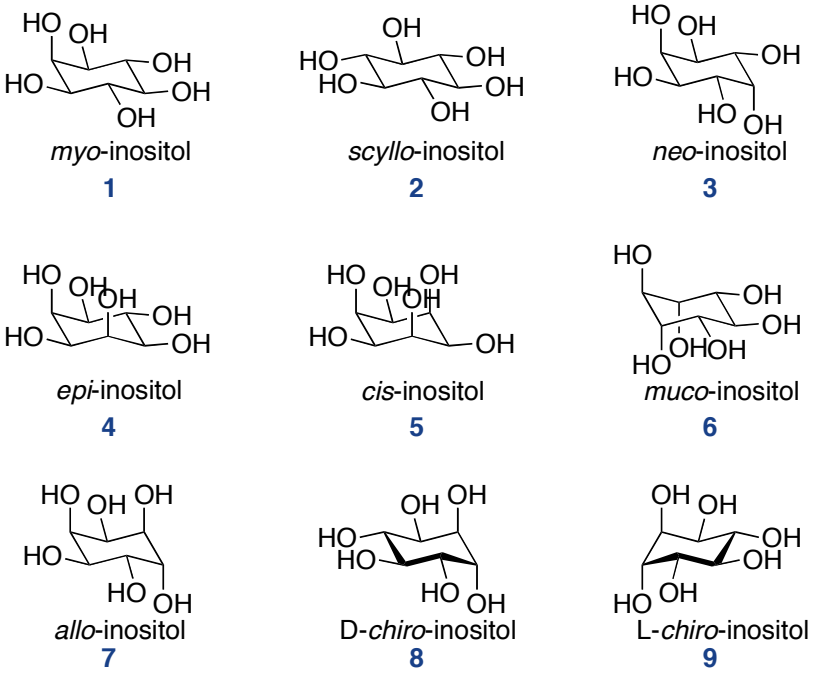


Figure 1.1. All nine possible stereoisomers of inositol.

myo-Inositol is a *meso* compound meaning that it is an achiral member of a set of diastereomers that also possesses chiral members. This property causes confusion when naming *myo*-inositol derivatives, using IUPAC rules, in some early publications. Different substitutions across the plane of symmetry sometimes changed the priorities of the substituents, thus causing the compounds to change between D- and L-numbering. Therefore, in order to bring some continuity to the naming, a relaxation of the lowest-locant rule was recommended, allowing all biologically related *myo*-inositols to possess the D-numbering.[1] Even though this allowed greater consistency within the scientific community, the numbering of the atoms in *myo*-inositol can still be confusing; therefore a useful mnemonic known as 'Agranoff's turtle' can be used to avoid uncertainty.[2,3]

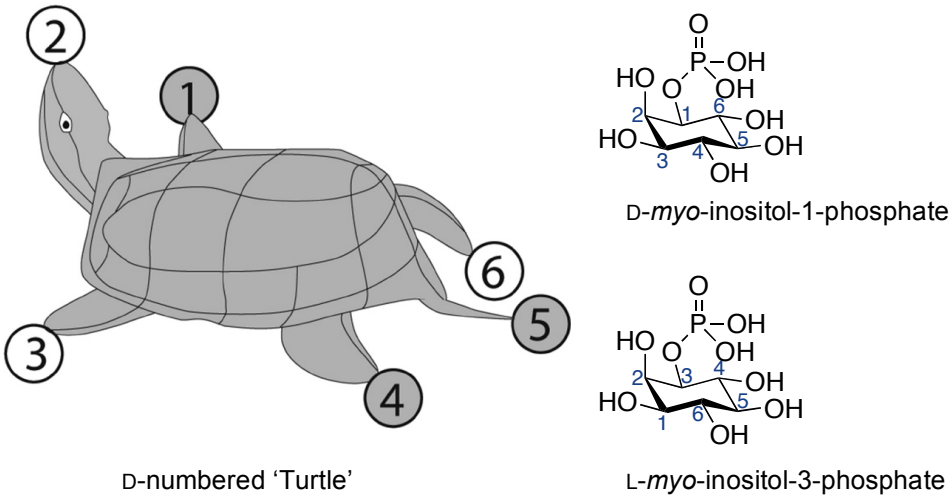


Figure 1.2. Bernard W. Agranoff's turtle mnemonic for the numbering of *myo*-inositol compounds and the structures of D-*myo*-inositol-1-phosphate and L-*myo*-inositol-3-phosphate.[3]

In the mnemonic, the turtle's head always represents the axial 2-position hydroxyl group (**fig. 1.2**). When using the D- numbering system one counts anticlockwise. Thus the head is the 2-position, the front left flipper is the 3-position, the front right flipper is the 1-position, the rear flippers are the 4- and 6-positions and the tail represents the 5-position. If one were to use the L-numbering system, one would count clockwise, thus the front right flipper would be the 3-position and the front left flipper would be the 1-position etc.

1.2. History of *myo*-inositol and introducing the phosphoinositides

myo-Inositol was first isolated from cardiac muscle tissue in 1850 by Scherer,[4] but little progress regarding its purpose or function within the body was made until 1942, when inositol-containing phospholipids were discovered in the brain.[5] At the time it was widely considered that phospholipids were purely inert structural components of membranes. However, a series of papers by Lowell and Mabel Hokin between 1953 and 1964, implicated phosphoinositides (PI) in receptor stimulated lipid turnover; a process that became known as the 'PI effect'.[6-11] Ultimately, this work paved the way for the seminal discovery published in 1983, by Berridge and co workers, that highlighted D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] as a key second messenger molecule responsible for the release of Ca^{2+} from intracellular stores.[12] This discovery triggered considerable interest in the study of inositol phosphates and their role in signalling pathways.[11,13,14]

It is now known that Ins(1,4,5)P_3 is formed by the action of phospholipase C (PLC) on PI, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2 **10**).[15] PtdIns(4,5)P_2 is part of a family of phosphoinositides, that regulate an increasingly complex network of cell signalling pathways concerned with an array of important cellular processes (**fig. 1.3**).[13,16,17]

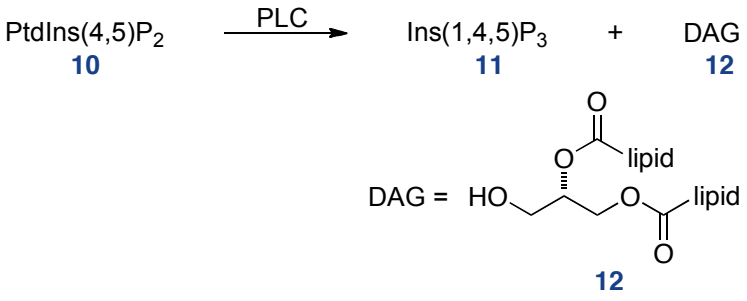
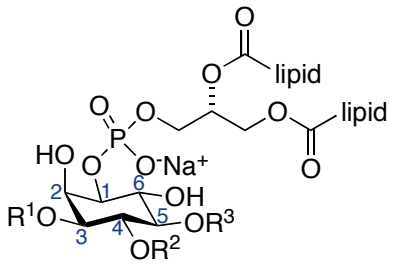


Figure 1.3. Enzyme catalysed hydrolysis of PtdIns(4,5)P_2 to give second messengers Ins(1,4,5)P_3 and DAG.

The PIs form a minor component of membrane phospholipids, and are bound to the membrane through their diacylglycerol (DAG) unit (**fig. 1.4**), itself an important second messenger,[18] which is attached to the 1-position phosphate. At present, there are eight known phosphoinositides (**fig. 1.4**), each bears the *myo*-inositol ring, with a cell membrane bound phosphatidyl moiety attached at the 1-position. They then vary in the amount and position of phosphorylation at the 3- 4- and 5-position hydroxyl groups.



	Hydroxyl substitution	Compound Name
13	$R^1=R^2=R^3=H$	PtdIns
14	$R^1=PO(OH)_2$, $R^2=R^3=H$	PtdIns(3)*P*
15	$R^2=PO(OH)_2$, $R^1=R^3=H$	PtdIns(4)*P*
16	$R^3=PO(OH)_2$, $R^1=R^2=H$	PtdIns(5)*P*
17	$R^1=R^2=PO(OH)_2$, $R^3=H$	PtdIns(3,4)P_2
18	$R^1=R^3=PO(OH)_2$, $R^2=H$	PtdIns(3,5)P_2
10	$R^2=R^3=PO(OH)_2$, $R^1=H$	PtdIns(4,5)P_2
19	$R^1=R^2=R^3=PO(OH)_2$	PtdIns(3,4,5)P_3

Figure 1.4. Structure of the eight naturally occurring PIs. The lipid side chains vary depending on the source of the PI.[19,20]

PIs are thought to mainly exert their activity through binding of their head groups to specific structural protein domains. The PIs both regulate the function of integral membrane proteins, as well as induce membrane translocation of cytoskeletal components, and cell signalling proteins.[13,16,17]

The site and degree of phosphorylation often allows for a remarkable selectivity in binding of these compounds with their respective effector proteins. For example, the general receptor for phosphoinositides 1 (GRP1) binds PtdIns(3,4,5)P_3 **19** strongly with $K_d \approx 100$ nM, but binds PtdIns(3,4)P_2 **17** and PtdIns(4,5)P_2 **10** with about 2-3 orders of magnitude lower affinity.[21]

The subcellular concentration and distribution of the different PIs is strictly mediated by a variety of enzymes, such as phospholipases, lipid kinases, and lipid phosphatases.[16] These enzymes are located in different cell membranes and organelles, this, coupled with the intrinsic selectivity each PI expresses for its effector protein, allows for extraordinary spatio-temporal control of PI

concentration (**fig. 1.5**); which is presumably why the PIs are so ubiquitous within many important cellular events.

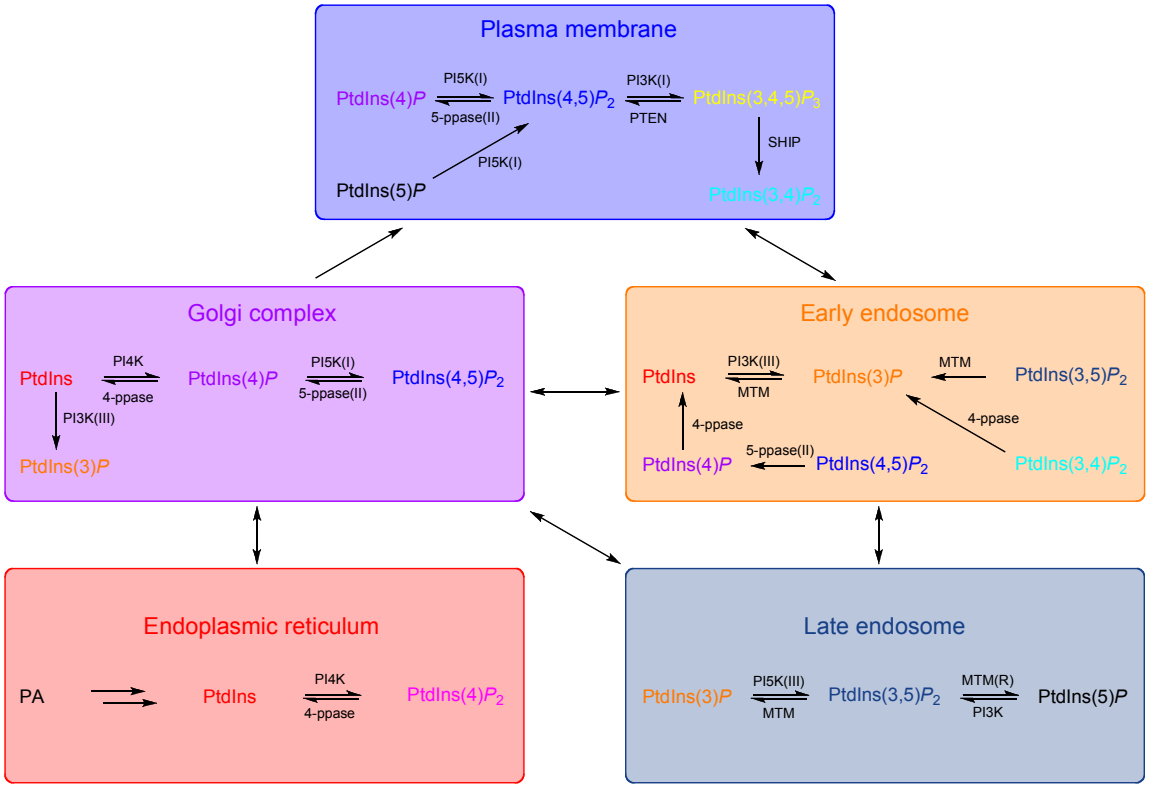


Figure 1.5. Subcellular localisations of the PIs. The PI colour matches its predominant locaton within the cell. Examples of the various enzymes that convert each PI are given.

Phosphatidyl inositol (PtdIns **13**) constitutes less than 15% of all the phospholipids within a eukaryotic cell, and is synthesised mainly at the endoplasmic reticulum. The phosphorylated derivatives of PtdIns (PtdInsP_n) typically exist in about one order of magnitude lower abundance. PtdIns(4)*P* **15** and PdtIns(4,5)P_2 **10** comprise the bulk of the phosphoinositides, with PtdIns(4,5)P_2 being the central precursor for the important second messengers Ins(1,4,5)P_3 **11** and DAG **12**. However, PtdIns(4,5)P_2, which resides mainly in the plasma membrane (**fig 1.5**), acts as a messenger in its own right playing an important role in a number of processes such as exo- and endocytosis, potassium channel regulation, and cytosketetal organisation.[16,22]

The action of the type I phosphoinositide-3 kinase [PI3K(I)] phosphorylates the 3-position of PtdIns(4,5)P_2 **10** to form PtdIns(3,4,5)P_3 **19**.[23] PtdIns(3,4,5)P_3 is normally present in negligible amounts when cells are in a rested state, but increases significantly in response to growth factor stimulation. PtdIns(3,4,5)P_3 along with PtdIns(3,4)P_2 **17**, are again most abundant in the plasma membrane (**fig 1.5**); they act as second messengers in signalling pathways associated with cell growth, cell survival and metabolism.[13]

PtdIns(3)*P* **14** is formed primarily by the action of type III phosphoinositide-3 kinase [PI3K(III)] on PtdIns. It is concentrated mainly in the endosome (**fig 1.5**) and participates in most aspects of endosomal activity, such as membrane fusion, interaction with the cytoskeleton, signalling and motility.[16,22] PtdIns(3)*P* can undergo phosphorylation by phosphoinosidite-5 kinase (PI5K) to form PtdIns(3,5)P_2 **18**, which is found mainly in the late endosome, and is thought to trigger vesicle budding.[24]

PtdIns(4)*P* **15** is concentrated at the Golgi complex (**fig 1.5**), its function is less well characterised, but a deficiency of this PI effects the Golgi's structure and function.[16] PtdIns(4)*P* is also thought to be the principal source of PtdIns(4,5)P_2.

There is very little PtdIns(5)*P* **16** present in cells and its location and function remain poorly characterised.[16]

1.3. PtdIns(3,4,5)P_3 and the PI3K/PKB pathway

Our interest in the PIs was at first centred around the study of PtdIns(3,4,5)P_3, and more specifically its role within the PI3K / protein kinase B (PKB) signalling cascade. Initially, looking at the interaction between PtdIns(3,4,5)P_3 and PKB. PtdIns(3,4,5)P_3 targets it's effector proteins by binding to specific structural domains within the protein. Its principal target is the plekstrin homology (PH) domain, although it is known to interact other non-catalytic sub-domains such as phosphotyrosine-binding (PTB) domain and phox homology (PX) domain.[13] The PH domain is the 11th most abundant domain class in the human genome.[25] It is a stretch of between 100-120 amino acids, and although there is very little sequence homology between the PH domains of different proteins, they all

convey a conserved structural fold.[26,27] This fold consists of a seven-stranded β-sandwich with one end capped with a C-terminal α-helix; the other end consists of three variable loops that interconnect the different β strands.

The PI3K/PKB signalling cascade is represented in **fig. 1.6**.[28] As was previously mentioned, PtdIns(3,4,5)P_3 concentrations exist at a very low level when the cells are at rest, however, stimulation of the cells by external growth factors lead to increases in PtdIns(3,4,5)P_3 concentrations of between 2- and 100-fold, through the action of type I PI3K on PtdIns(4,5)P_2. This increase leads to the accumulation of PKB to the plasma membrane, through the selective binding of the PKB-PH domain to the PtdIns(3,4,5)P_3 head group. Two other proteins, phosphoinositide dependant kinases 1 and 2 (PDK 1 and 2), also bind to PtdIns(3,4,5)P_3 *via* their PH domains, which brings them in close proximity to PKB. PDK 1 and 2 phosphorylate PKB, which results in several downstream events associated with cell survival and proliferation, growth, translation and transcription.

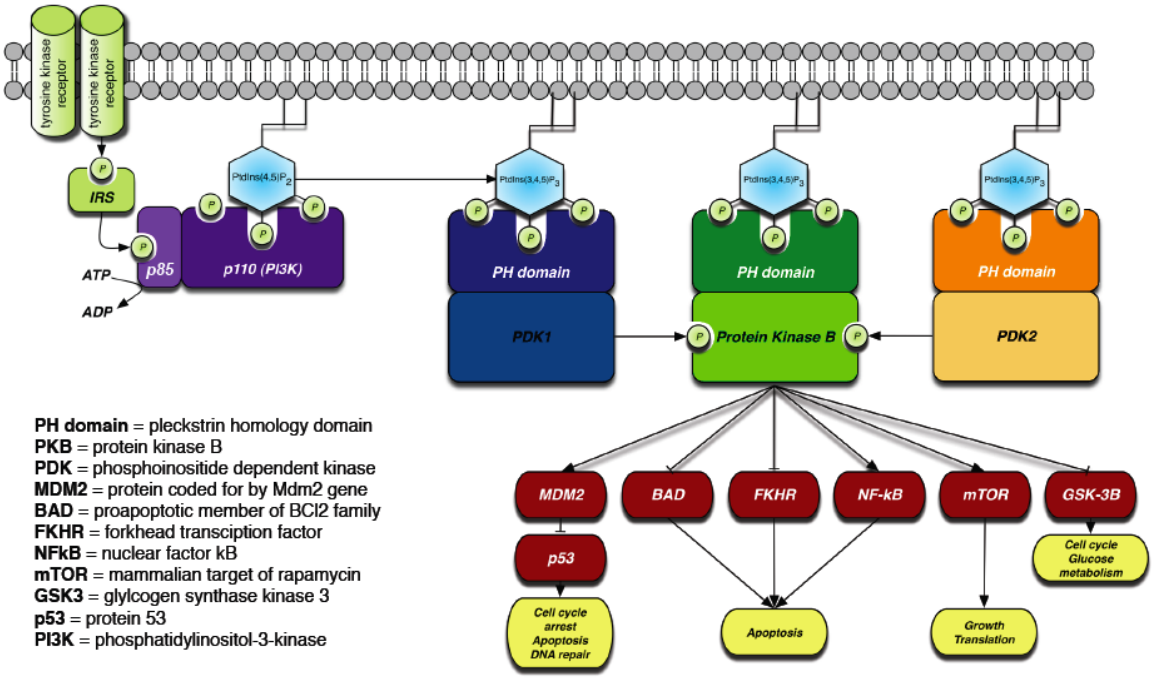
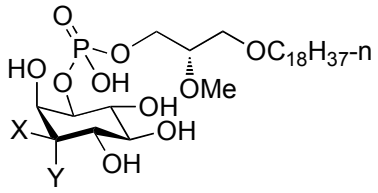


Figure 1.6. Outline of the PI3K signalling pathway and PKB activation of down stream events.[28]

Significantly, upregulation of the PI3K/PKB signalling pathway has been shown in numerous human cancer types.[28] As a consequence this upregulation promotes greater PKB activity thus allowing an increase in the processes associated with cell survival and DNA repair, and a decrease in processes associated with cell apoptosis. Thus allowing the cancer cells to subsist and thrive within tumours, which are intrinsically stressful environments for cells to live in. The central role that the PtdIns(3,4,5)P_3-PKB PH domain interaction plays within these important cellular events linked with cancer, makes it an attractive proposition to develop an inhibitor of PKB that will disrupt that PtdIns(3,4,5)P_3-PKB PH domain interaction. These inhibitors might provide an effective therapeutic for the treatment of several types of human cancer; Kozikowski and co-workers subsequently provided a proof of this concept.[29,30] They synthesised a range of 3-position modified PtdIns-lipid ether analogues (**fig. 1.7**) and showed that these inhibited PKB with low μM activity, causing a reduction in the growth of colon and breast cancer cell lines in mouse studies. They also suggested that the active components were likely to be the 4- and 5-position phosphorylated versions of their inhibitors, through the *in vivo* action of PI4K and PI5K. They have yet to synthesise the phosphorylated analogues to confirm this hypothesis.



20 X=H, Y=H
21 X=H, Y=CH_2OH
22 X=CH_2OH, Y=H

Figure 1.7. Kozikowski's anti-cancer 3-position modified PtdIns-lipid ether analogues.[29,30]

Previous work conducted within the group, by Nemeth,[31] was aimed at the design and synthesis of PtdIns(3,4,5)P_3 analogues, as potential inhibitors of the PKB PH domain. These analogues were proposed to act as small molecule probes for

the further study of the PI3K / PKB pathway. Nemeth successfully synthesised of a number of analogues based on Ins(1,3,4,5)P_4, which is the head group of PtdIns(3,4,5)P_3 (**fig. 1.8**), and they were subsequently submitted for biological analysis. The results showed that both sets of analogues **24** – **29** (**fig. 1.8**) exhibit no binding affinity for the PKB PH domain.

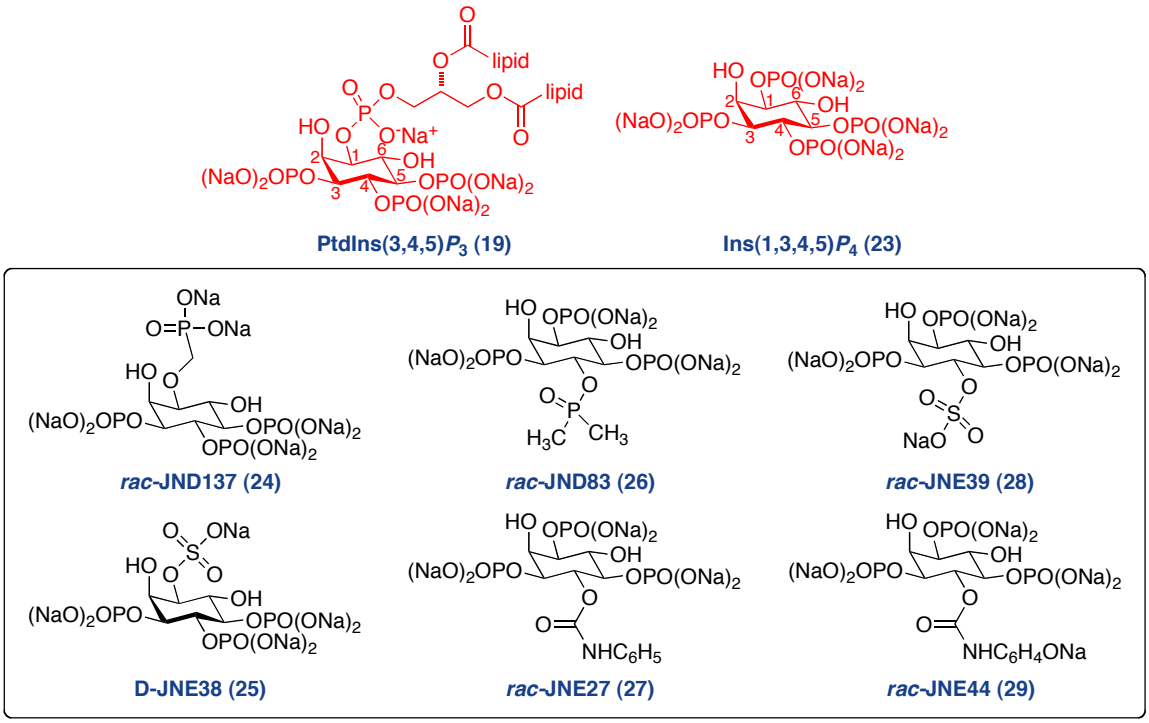


Figure 1.8. The structures of PtdIns(3,4,5)P_3, Ins(1,3,4,5)P_4, and Nemeth's Ins(1,3,4,5)P_4 derivatives **24**-**29**.

However, in addition to PKB, these analogues were also tested for their binding affinity towards the general receptor for phosphoinositides 1 (GRP1) PH domain. It was found that the 1-position modified Ins(1,3,4,5)P_4 derivatives, **24** and **25**, exhibited binding at concentrations over ~0.1 μM, and that derivatives modified at the 4-position (**26**-**29**) expressed no binding affinity for the GRP1 PH domain. This observed selectivity prompted us to postulate the potential for expanding the project, broadening the scope to include the design of inhibitors for the other PH domain containing proteins that interact with the phosphoinositides. In this way, we might be able to develop a tool kit of PH domain selective inhibitors for the study of not only the PKB signalling cascade (which is linked with cancer),[28] but also for the signalling pathways associated with GRP1, Bruton's tyrosine kinase

(Btk), and ARF nucleotide-binding-site opener (ARNO) all of which bind to PtdIns(3,4,5)P_3 through their respective PH domains.[32]

GRP1 has been shown to be involved in insulin signalling and cytoskeletal rearrangements.[11,33] Btk plays an important role in lymphocyte development; but importantly, X-linked agammaglobulinemia (XLA), which is a hereditary immunodeficiency disease, is caused by a mutation in Btk.[34,35] ARNO is involved in cytoskeletal rearrangements and membrane trafficking.[36] Selective inhibitors targeted specifically for their PH domains will be able to shed light on a number of important cellular and disease linked processes.

This dissertation describes the development, synthesis and biological analysis of PtdIns(3,4,5)P_3 analogues. The development of a robust and flexible synthetic route to PtdIns(3,4,5)P_3 analogues is certainly one of the key features when attempting to synthesise a range of these compound. But in the first instance, it is vital to impliment a logical design of potential inhibitors. Our approach towards our initial range of compounds will be a very minimalist one. Our structures will be based around the head group of PtdIns(3,4,5)P_3, Ins(1,3,4,5)P_4 (known to bind stongly with the PH domains of PKB, GRP1 and Btk),[21,37,38] and will initially bear only a single substitution of a phosphate group with an isosteric phosphate replacement, retaining as much structural similarity to Ins(1,3,4,5)P_4 as possible, without being identical (**fig. 1.9**).

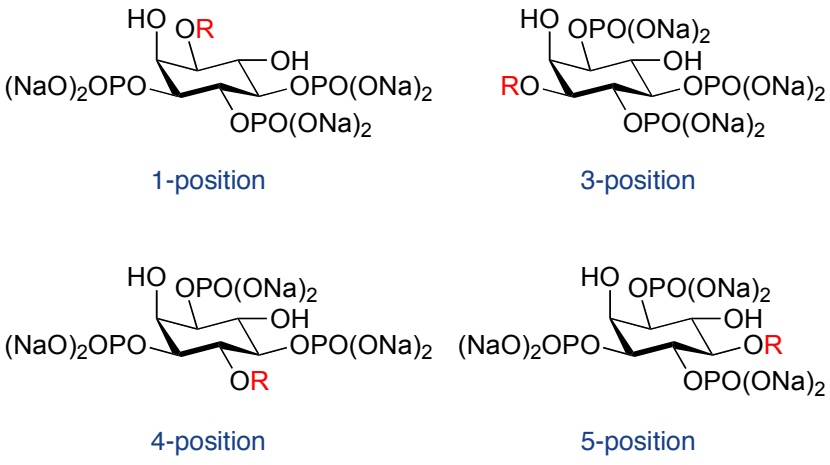


Figure 1.9. General target structures of Ins(1,3,4,5)P_4 derviatives. R = phosphate bioisosteric replacement.

The synthesis of a range of Ins(1,3,4,5)P_4 derivatives with a mono-substitution of each phosphate, will allow us to ascertain, in detail, the importance that each phosphate possesses for each PH domain's recognition of PtdIns(3,4,5)P_3. Then we will be able to use our findings in the design and synthesis of a new generation of analogues that will hopefully be more targeted for their respective PH domains. Thus, the design of the PH-domain ligands becomes a problem of finding good phosphate bioisosteres. Herein, the range of moieties that have been used as phosphate mimics is critically reviewed.

1.4. Phosphate Bioisosteres Review

It is clear that the addition and removal of the phosphate group in phosphoinositide and inositol phosphate function, controlled by the vast array of inositol kinases and phosphatases, plays a hugely important role in the regulation of many cellular processes. The importance of the phosphate group, however, extends far beyond its role in inositol-containing compounds. It is one of the major structural constituents of DNA and RNA, and is the cellular unit of energy in ATP, the main energy source of innumerable biological processes. Indeed, the reversible phosphorylation of proteins to transmit signalling information is one of the most ubiquitous and fundamental processes within the cell.

Protein kinases bind ATP to phosphorylate their specific substrates at certain amino acids (typically serine, threonine or tyrosine). In medicinal chemistry, inhibitors of protein kinases commonly target the ATP binding domain; they, however, pay little attention to the phosphate group, as the phosphorylated products usually display little affinity for the kinase domain and diffuse away. So in this case, the phosphate moiety only plays the role of passenger and is not generally involved in the recognition of either ATP or the substrate protein. However, in the case of phosphinositide second messenger activity, the phosphate is crucial for substrate recognition, and so for inhibitors that target the protein – phosphoinositide interaction, consideration of the phosphate moieties

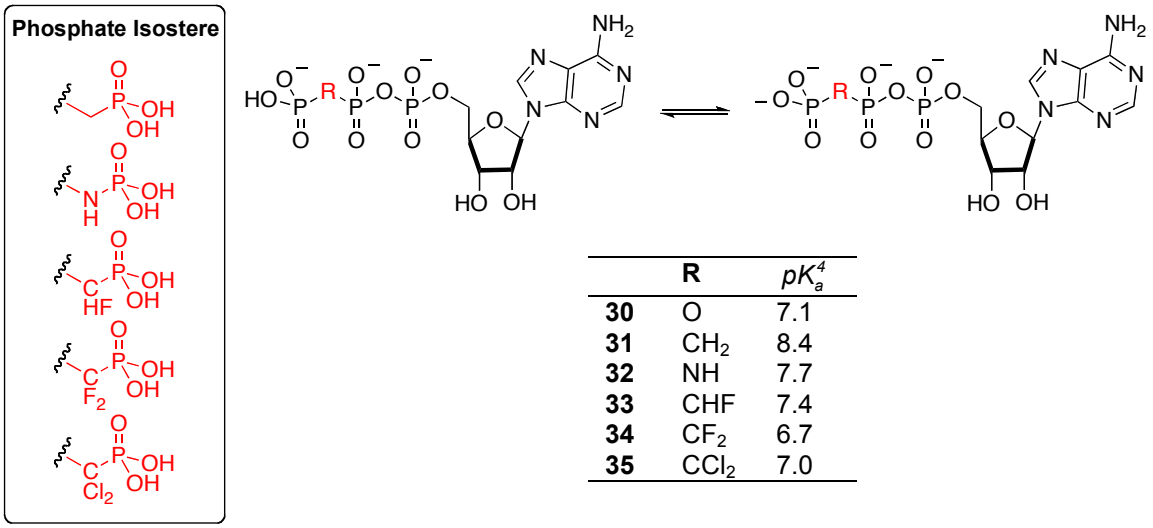
must be paramount to their design. This recognition of a non-catalytic domain by a phosphorylated species is not restricted to the PH domain – phosphoinositide interactions. Another important example of this recognition is of phosphotyrosine (pTyr) residues with Src homology 2 (SH2) and pTyr binding (PTB) domains, and is the target of a many inhibitors bearing isosteric phosphate replacements.[39,40] The amount and significance of phosphate recognition within so many important cellular processes, renders the development of inhibitors that target phosphate-recognising enzymes is an increasingly vital and diverse area of research.

To design an exogenous ligand that targets a phosphate recognition domain (such pTyr) with high selectivity and recognition, a simple approach might be to incorporate a phosphate group to achieve the desired effect. However, the major disadvantage of an approach such as this is the prevalence of *in vivo* phosphatases, which will likely hydrolyse the phosphate group, diminishing the ligand's activity. In addition, the dianionic nature of the phosphate at physiological pH, might compromise the bioavailability of the inhibitor. As a result, isosteric phosphate replacements have been used in order to avoid these problems. These replacements are usually designed to be hydrolytically stable, often bearing reduced or even neutral charge.

The following is a review of structures designed to mimic the role of a phosphate group. However, it is beyond the scope of this review to highlight 'good' isosteres over 'bad' isosteres, as all the examples given are from a variety of different studies, for a variety of different targets, and for a variety of different purposes. The aim of this review is to indicate some of the different types of isostere used, based on broad structural characteristics, highlighting the logic behind some of the modifications used and contex (where appropriate) in which they were used.

1.5. Phosphorus-based Isosteres

A logical start point, when designing phosphate replacements, is to retain the phosphorus atom and modify the substituents (oxygen) that surround it. The first and most commonly used, phosphate isoster is the methylene phosphonate, in which the bridging oxygen is replaced with a methlyene (CH_2) group. The reduced electronegativity, due to the carbon atom, lowers the acidity of the group, resulting in differing ionisation states at physiological pH. In addition to slightly inceasing the pK_a from 7.1 to 8.4 (**figure 1.10**), the inclusion of carbon also removes a hydrogen bond acceptor. This group was successfully use in the mid 1960s, by Myers and co-workers,[41,42] whereby they synthesised a methylene phosphonate derivative of ATP (AMPPCP, **31**, [**fig. 1.10**]), showing that it was both metabolically stable and acted as an ATP inhibitor. This analogue possessed certain drawbacks, as it was a poor agonist at some ATP receptors, and as a result, triggered research to improve the utility of ATP analogues.



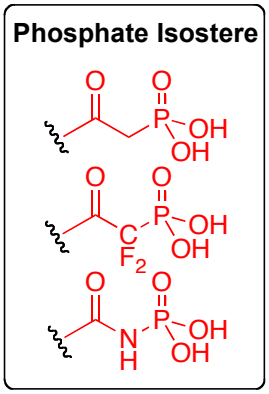
	R	pK_a^4
30	O	7.1
31	CH_2	8.4
32	NH	7.7
33	CHF	7.4
34	CF_2	6.7
35	CCl_2	7.0

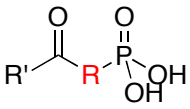
Figure 1.10. ATP analogues **31**-**35** bearing terminal phosphate isosteres and their respective pK_a values.[41-44]

One of the first logical changes to the methylene phosphonate structure, was to substitute the terminal bridging oxygen with an NH to give AMPPNP **32** (**fig. 1.10**),[43] this alteration increases the pK_a^4 from 7.1 for **30** to 7.7 for **32**. This modification was met with moderate success in early studies but the highly labile

P-N-P linkage meant it suffered rapid hydrolysis, and thus limiting its use. In order to retain the hydrolytic stability of AMPPCP and introduce the required electronegativity at the terminal bridging atom, derivatives bearing P-CCl_2-P (**35**), P-CFH-P (**33**) and P-CF_2-P (**34**) were synthesised by Blackburn and co-workers (**fig. 1.10**).[44] These analogues showed pK_a^4 values close to that of the natural ATP, with the dichloro **35** pK_a^4=7.0, monofluoro **33** pK_a^4=7.4, and difluoro **34** pK_a^4=6.7, and subsequently the hydrolytically stable difluoromethylene phosphonate is now used widely as an isosteric phosphate replacement.

More recently, Grimes and co-workers synthesised a range of acyl phosphate mimics as inhibitors of PlsY,[45] an essential acyltransferase for the biosynthesis of phosphatidic acid in bacteria. They showed that the methylene phosphonate analogue **36** (**fig. 1.11**) exhibited activity against *B. anthracis* (*Ba*PlsY1) with an IC_{50} = 90 μM. The more acidic difluoromethylene phosphonate analogue of **37** (**fig. 1.11**), was more potent (IC_{50} = 50 μM) against *Ba*PlsY1 and was also highly potent against *S. pneumoniae* (*Sp*PlsY) with an IC_{50} = 39 μM. In addition to the methylene and difluoromethylene containing isosteres, Grimes also synthesised the phosphoramide **38** (**fig. 1.11**). This analogue was the most potent against *Sp*PlsY with an IC_{50} = 11 μM, which was four times more potent than the equivalent methylene phosphonate analogue. The substitution of O with NH will give the isostere a slightly increased pK_a compared to that of the native phosphate. However, the isostere retains the hydrogen bond acceptor activity, compared with the methylene phosponate, as well as contributes an additional hydrogen bond donor, in the form of N-H.



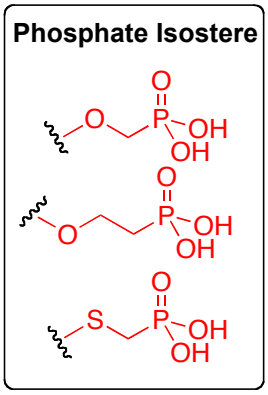


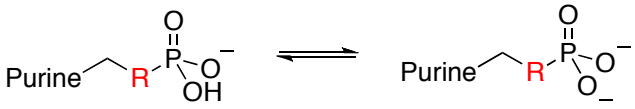
	R
36	CH_2
37	CF_2
38	NH

R' = various aliphatic side chains

Figure 1.11. Acyl phosphate mimics **36**-**38** bearing phosphate isosteres.[45]

A study conducted by Kim and co-workers[46] into some highly potent anti-viral agents explored the effect of methylene phosphonate, the extended, ethylene phosphonate and the isoelectronic thiomethylene phosphonate (**fig. 1.12**). Comparing the second dissociation constants they found that the methylene phosphonate **39**, ethylene phosphonate **40** and the thiomethylene phosphonate **41** all exhibited pK_a^2 values very close to one another (6.52, 6.76 and 6.59, respectively). It is interesting to note that **39** was the only active compound of the three and in addition to this, ethylene phosphonate **40** did not undergo phosphorylation by bovine brain guanylate, whereas methylene phosphonate **39** did.





	R	pK_a
39	OCH_2	6.52
40	OCH_2CH_2	6.76
41	SCH_2	6.59

Figure 1.12. Purine based analogues **39**-**41** bearing phosphate isosteres and their respective pK_a values.[46]

An interesting evolution of the methylene phosphonate isostere, was highlighted by Shakespeare in 2001.[39,47] He incorporated a diphosphonomethyl isostere **42** (**fig. 1.13**) as a pTyr mimetic, for the inhibition of the SH2 domain of Src. This highly charged isostere, showed a sevenfold increase in affinity, compared to that of the native pTyr analogue. This isostere is one of the only examples of pTyr substitution that demonstrates an increased affinity over pTyr, however, its highly charged nature will undoubtedly give the isostere poor bioavailablity.

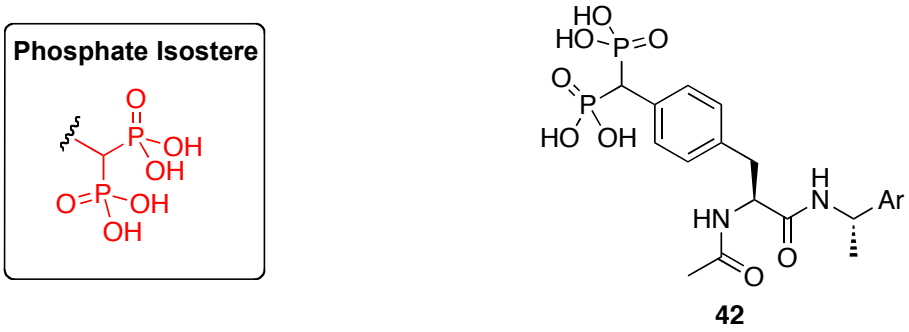


Figure 1.13. pTyr mimetic bearing phosphate isostere that displays higher affinity that the natural phosphate.[39,47]

1.6. Monocharged Phosphorus-based Isosteres

Instead of changing the bridging oxygen as with those mentioned above, another logical modification is to change one of the acidic or charge-bearing oxygen atoms. van Boom and co-workers successfully demonstrated the utility of this approach,[48] by the synthesis of a racemic methyl phosphonate analogue of Ins(1,4,5)P_3 **43** and a racemic difluoromethyl phosphonate analogue of Ins(1,3,4,5)P_4 **45** (**fig. 1.14**). These isosteric phosphate replacements bear only a single charge and, in the case of the methylphosphate, a reduced pK_a. Their biological results indicated that **43** acted as an antagonist for Ins(1,4,5)P_3 stimulated Ca^{2+} release, indicating that the methylphosonate moiety was tolerated by Ins(1,4,5)P_3 receptors, no biological discussion was given for analogue **45** (**fig. 1.14**). Recently, work within the Conway group by Aslam and

Keddie,[49] successfully synthesised **44** in an enantiomerically pure fashion and demonstrated that it is an Ins(1,4,5)P_3 receptor antagonist.

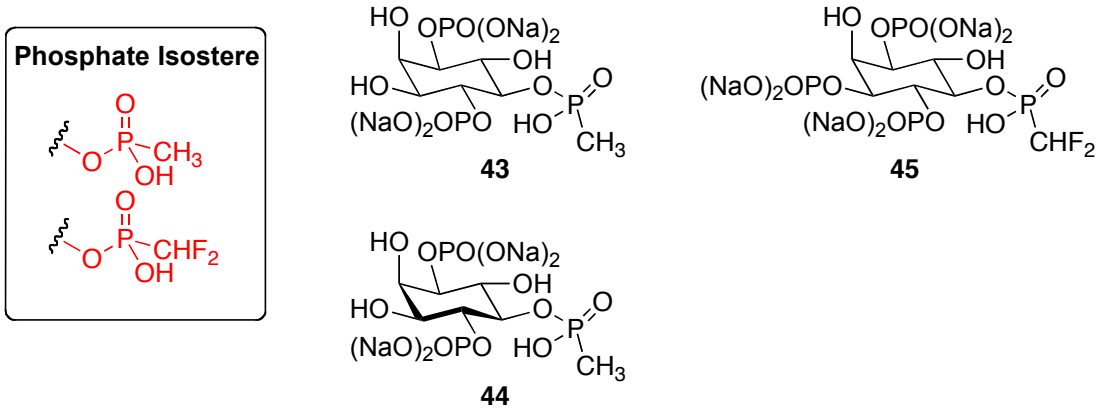


Figure 1.14. Methylphosphonate and difluoromethylphosphonate bearing Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 analogues.[48,49]

Raushel *et al.* demonstrated an interesting use of this methyl substitution.[50] They synthesised two methylphosphonamidate-bearing analogues, one on amino-glucose **46**, and the other on aspartic acid **47** (**fig. 1.15**). They demonstrated that these analogues where extremely potent inhibitors of two deacetylases belonging to the amidohydrolase superfamily. Sugar **46** showed inhibition of NagA at 34 nM, whereas amino acid **47** inhibited DGD at 460 pM. Strictly speaking the methyl phosphonamidates, are acting as mimics of the tetrahedral acetyl intermediate, during the enzymatic hydrolysis of an *N*-acylated substrate. However, this paper does demonstrate the potential for the group to be used as a true phosphate mimetic.

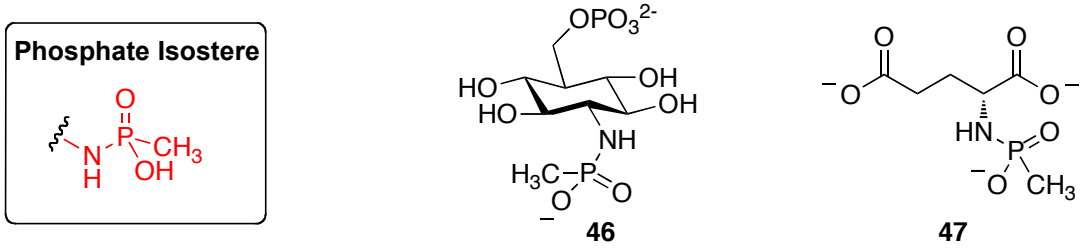


Figure 1.15. Methylphosphonate and difluoromethylphosphonate bearing Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 analogues.[50]

Burke and co-workers synthesised a range of macrocyclic tetrapeptides (**fig. 1.16**),[51] bearing different pTyr mimics as potent SH2 domain-binding antagonists. Their results showed that the hydrolytically stable methylene phosphonate derivative **48** exhibited the highest affinity (K_D = 1.47 nM). They also explored mono charged phosphorus base mimics **49** and **50**, varying the size of the side group installed. These compounds also displayed nanomolar affinities.

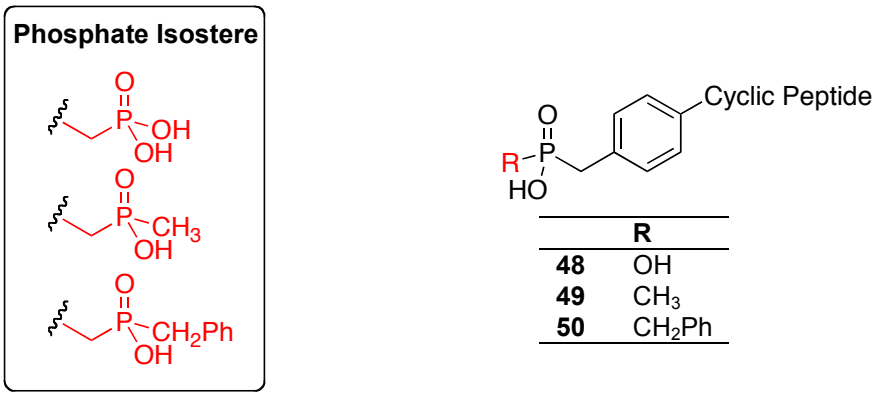


Figure 1.16. Cyclic peptide pTyr analogues **48**-**50** bearing phosphate isosteres.[51]

1.6. More Unusual Phosphorus-based Isosteres

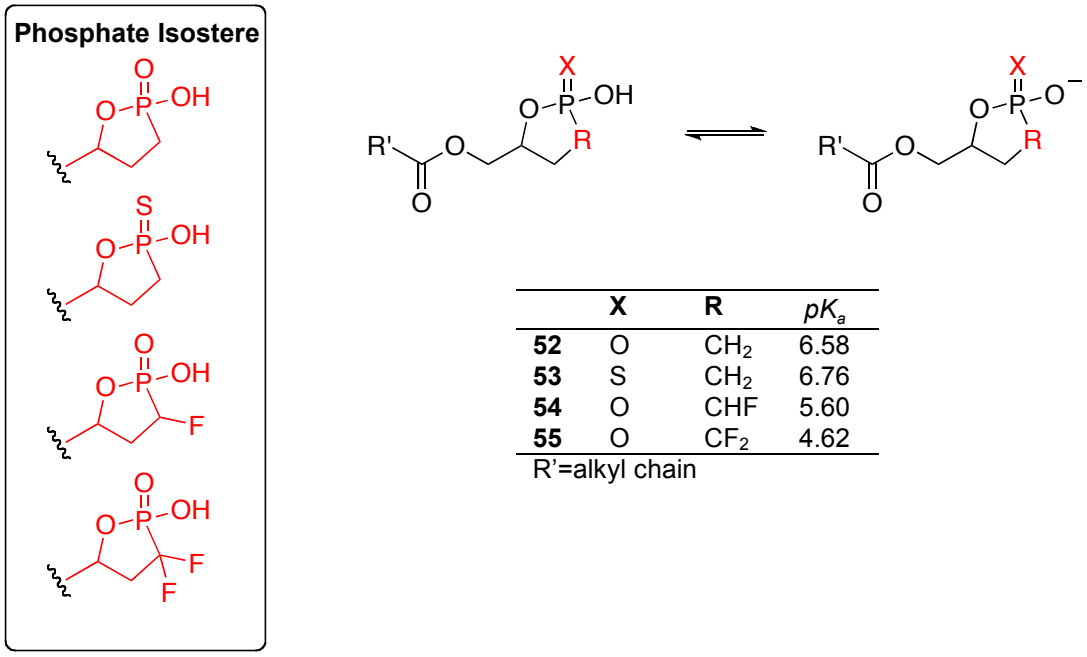
Another successful phosphorus-containing mimetic is the use of phosphorothioates. Phosphorothioates are phosphates in which one or more of the oxygon atoms have been replaced by an isoelectronic sulfur atom. This isostere has been put to good use in inositol chemistry. Potter *et al.* synthesised racemic Ins(1,4,5)P_3 with each phosphate replaced by a phosphorothioate **51** (**fig. 1.17**).[52] **51** was subsequently shown to stimulate Ca^{2+} release *via* binding to Ins(1,4,5)P_3 receptors. Not only was agonist activity observed, but the phosphorothioates also proved to be more metabolically stable than Ins(1,4,5)P_3.

51

Figure 1.17. Ins(1,4,5)P_3 analogue **51** bearing phosphorothioate replacements for each phosphate.[52]

Introducing metabolic stability to phosphate isosteres has always been of paramount importance, and so has been approached in a variety of ways. Recently, Prestwich and co-workers have described a novel set of metabolically stabilised analogues of lysophosphatidic acid (LPA)[**fig. 1.18**].[53] LPA is an important phospholipid involved in a diverse array of biological processes, such as platelet aggregation, cell survival, and cell migration. They synthesised a range of cyclic phosphonates **52**-**55** (**fig. 1.18**), exploring the isoelectronic nature of cyclic methylenephosphonate **52** and cyclic methylenephosphonothioate **53**. In addition to this, they also explored the effect of pK_a by the synthesis of cyclic mono- and difluoromethylenephosphonates **54** and **55**. They measured pK_a values (**fig. 1.18**) of each analogue and found that the methylenephosphonate **52** and the methylenephosphonothioate **53** values are well matched (pK_a = 6.58 and 6.76, respectively) and that the monofluoromethylenephosphonate **54** and the difluoromethylenephosphonate **55** exhibit reduced pK_as (5.60 and 4.62, respectively). The biological activity expressed by each isostere, however, was quite intriguing, in that all analogues showed strikingly different results. No activity for the three G-protein coupled receptors (LPA_1, LPA_2 and LPA_3) was exhibited by cyclic methylene phosphonate **52**. However, the cyclic methylene phosphonothioate **53** and cyclic monofluoromethylenephosphonate **54** both exhibited anatagonist activity towards LPA_1 and LPA_3. But the addition of an extra fluorine atom in the cyclic difluoromethylene phosphonate **55** showed a flip in activity, being weak agonist activity towards LPA_2 and LPA_3. These examples clearly indicate that there is intricate complexity in the nature of receptor and

ligand interactions that stretch beyond the effects that isosteres express on compound conformation and pK_a values.



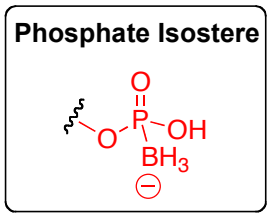
	X	R	pK_a
52	O	CH_2	6.58
53	S	CH_2	6.76
54	O	CHF	5.60
55	O	CF_2	4.62

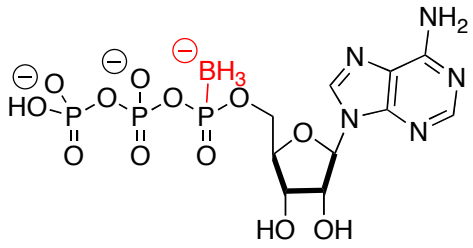
R'=alkyl chain

Figure 1.18. Cyclic phosphatidic acid analogues **52**-**55**, and their respective pK_a values; anogists of lysophosphatidic acid receptors.[53]

An interesting and unusual phosphate isostere, has recently been demonstrated by Fischer *et al*.[54] They synthesised a range of ATP analogues bearing a 1-boranophosphate **56** (**fig. 1.19**). These compounds were shown to behave as $P2Y_1$ receptor agonists, but also displayed remarkable hydrolytic stability under physiological and gastric juice pH values at 37 ºC ($t_{1/2}$ 1395 h and $t_{1/2}$ 59 h, respectively). They applied this isostere to the synthesis of dinucleoside polyphosphates (Np_nN).[55] Np_nNs are a diverse group of biomolecules,[56] their function is still largely unknown although they have been implicated in DNA replication.[57,58] The Np_nNs extracellular effects are better characterised, having been shown to stimulate NO release,[59] and inhibit platelet aggregation.[60] In addition to the continuing investigations of Np_nNs biological roles, Np_nN analogues are also undergoing clinical trials for a number of therapeutic targets.[61] However, some of the endogenous Np_nN analogues exhibit short *in vivo* half-lives, e.g. Up_4U $t_{1/2}$ = 50 min. Their Ap_3(β-B)A analogue **57** (**fig. 1.19**)

was a potent $P2Y_1$-R agonist and showed 40% and 59% slower hydrolysis compared to Ap_3A, when submitted to human nucleotide pyrophosphatase phosphodiesterases, NPP1 and NPP3, respectively.





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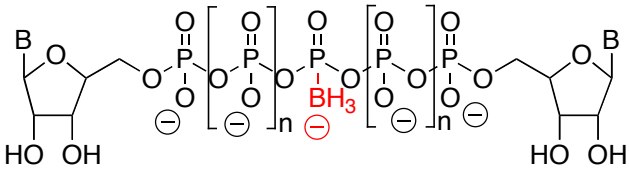


57

Figure 1.19. Borano triphospate analogue of ATP **56**, general structure of borano phosphate analogues of Np_nN' (n=0 or 1, B=A or U).[55]

1.7. Sulfur-based Isosteres

When considering phosphate isosteres that are not based around phosphorus, one of the most logical moves is to consider sulfur, which is the focus of a significant number of phosphate replacements. Like phosphorus, sulfur can exist in a number of stable oxidation states as well as possess different coordination numbers, giving sulfur great flexibility in synthesis. However, phosphorus is at its highest stable oxidation state [P(V)] in a phosphate and thus the sulfur based phosphate equivalents are also oxidatively saturated.

A simple and widely used sulfur-based phosphate isostere is the sulfate moiety. The sulfate ($pK_a \approx$ -3.0) is mono charged at physiological pH, but like a phosphate it is tetrahedral and contains multiple hydrogen bond acceptors. However, this brings with it drawbacks akin to phosphates, such as hydrolytic

instability and poor bioavailability. Despite these issues the sulfate is still a widely employed phosphate isostere.

A study by Larsen and co-workers, on a novel class of low molecular weight protein tyrosine phosphatase (PTP) inhibitors,[62] demonstrated that the sulfate **58** (**fig. 1.20**) was an excellent pTry mimetic, exhibiting 80% inhibition of PTP1. In this study the effects of non-charged sulfate analogues methylsulfonate **59** and trifluoromethylsulfonate **60** (**fig. 1.20**), were compared. However, work by Taylor *et al.* on the same target,[63] compared a different range of pTyr mimics. Their results showed that the difluoromethylenesulfonic acid isostere **62** exhibited a 1000-fold reduction in activity, compared to the difluoromethylenephosphonate isostere. It is interesting to note was that the methylenesulfonic acid **61** and the difluoromethylenesulfonic acid **62** showed remarkably similar activities, indicating that the fluorine atoms have little impact in this case. Further pTyr mimics, published by Seto and co-workers,[64] were installed on a short oligo-peptide and their inhibitory action towards two PTPs evaluated. Their methylene sulfonamide analogue **63** (**fig. 1.20**) inhibited *Yersinia* PTP with an IC_{50} of 370 μM.

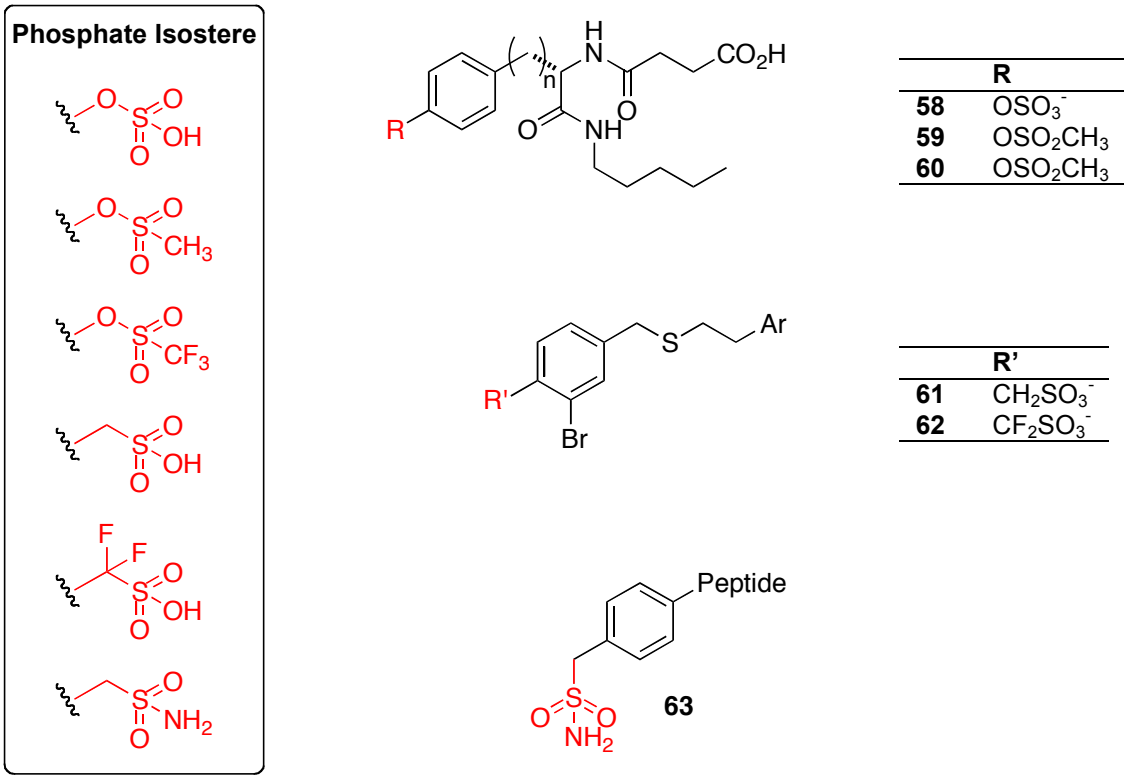


Figure 1.20. Sulfate-based pTyr mimics **58**-**63**.[62-64]

Early work by van Boeckel *et al.*,[65] as well as recent work by Prestwich *et al.*,[66] show the syntheses of novel Ins(1,4,5)P_3 dervatives in which both sulfate and sulfamate phosphate isosteres replace the phosphates of Ins(1,4,5)P_3, and a derivative **64**, **65** (**fig. 1.21**). It was subsequently shown that this much modification led to the compounds having no binding affinity for Ins(1,4,5)P_3 receptors.

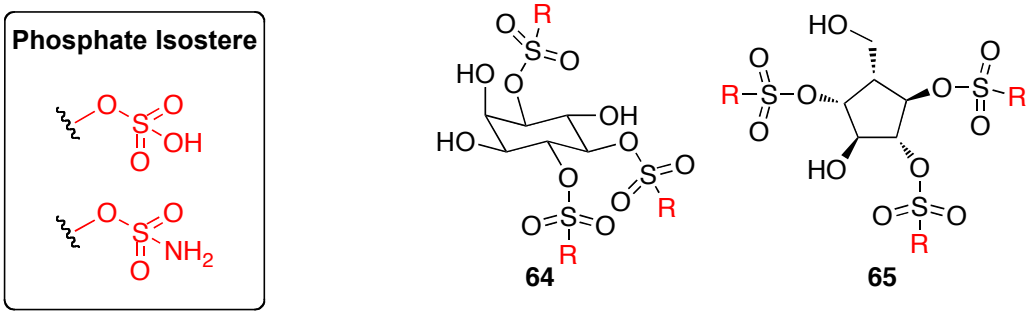
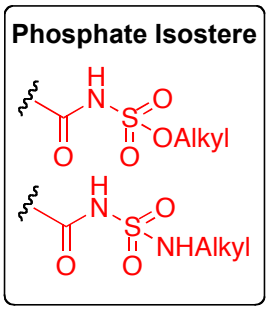
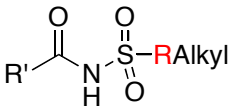


Figure 1.21. Sulfur-based Ins(1,4,5)P_3 mimics. **64**, **65** R=OH or NH_2.[65,66]

In the previous section, a comparison of the antimicrobial acylphosphate mimics **36**-**38** was made.[45] In addition to these compounds, Grimes also synthesised a range of analogues containing sulfur-based acylphosphate isosteres. In this paper she compared the effect of acyl sulfamate esters **66** and acyl sulfamide esters **67** (**fig. 1.22**). The sulfamate-bearing analogues, **66**, all showed good activity against *B. anthracis*, with IC_{50} values ranging from 35-65 μM. The sulfamide **67**, which bears an additional hydrogen bond donor (NH), exhibited lower activity (IC_{50} = 200 μM), but nonetheless is clearly well tolerated within their targets.





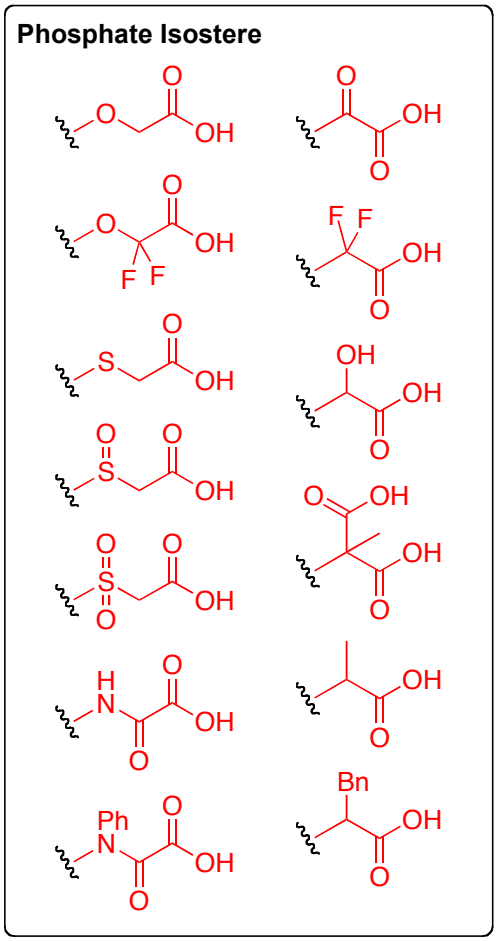
	R
66	O
67	NH

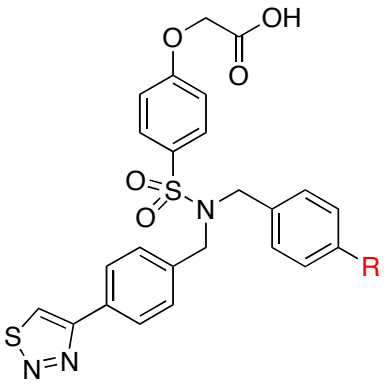
Figure 1.22. Sulfate-based acylphosphate mimics **66** and **67**.[45]

1.8. Carboxylate-based Isosteres

The difluoromethylene phosphonate is one of the most successful metabolically stable phosphate isosteres, its high affinity can be attributed to the closely matched pK_a values with the native phosphate as well as direct hydrogen bonding between the fluorines and the enzyme active site.[67] The major drawback is, however, its poor bioavailability. As a result attention has been drawn to the development of hydrolytically stable and more bioavailable phosphate isosteres. One of the most abundant areas researched is the use of carboxylate-based isosteric phosphate replacements, and this is the subject of the next part of this review.

As with many isosteric phosphate replacements, pTyr mimics have included a vast array of carboxylate derivatives. Grove and co-workers synthesised a large range of carboxylate based pTyr mimics in order to explore alternatives for their difluoromethylene phosphonate analogue, which displays excellent activity but poor bioavailability.[68] A list of their phosphate replacements is shown in **fig. 1.23**. A direct comparison of each carboxylate derivative purely as a phosphate replacement is meaningless in a study such as this, as the measure of effectiveness relies on many variables such as the substitutions on the phenyl ring and the conformations it can adopt within the receptor. However, what this paper highlights are the logical progressions used when trying to find a phosphate, or in this case, a methylene phosphonate, replacement. Included are groups such as the *O*-methylene carboxylate **68**, the *O*-difluoromethylene carboxylate **69**, and the *S*-methylene carboxylate **70**, these will all display similar spatial arrangements, but possess different pK_a values and electronic properties. In order to address possible steric constraints the truncated difluoromethylene carboxylate **76** was also synthesised. Extra hydrogen-bond acceptor character was introduced through the inclusion of sulfoxide **71**, sulfone **72**, and ketone **75**. Hydrogen-bond donor character was added through the addition of alcohol **77** and a mixture of hydrogen-bond donor and acceptor character was introduced through the inclusion of amide **73**. Aliphatic and aromatic side chains were also explored by the synthesis of compounds **74**, **79** and **80**; as well as a di-charged analogue in the form of the malonate **78**.

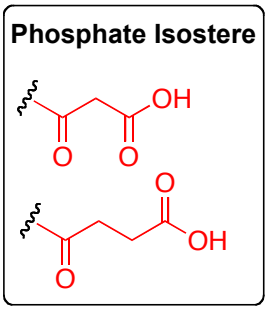


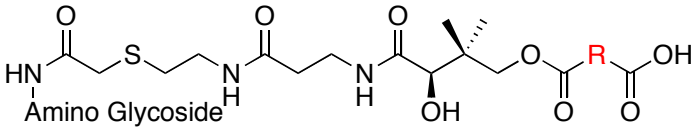


	R
68	OCH_2CO_2H
69	OCF_2CO_2H
70	SCH_2CO_2H
71	$S(O)CH_2CO_2H$
72	$SO_2CH_2CO_2H$
73	$NHC(O)CO_2H$
74	$NPhC(O)CO_2H$
75	$C(O)CO_2H$
76	CF_2CO_2H
77	$CH(OH)CO_2H$
78	$C(CO_2H)_2Me$
79	$CH(CH_3)CO_2H$
80	$CH(Bn)CO_2H$

Figure 1.23. Carboxylate-based pTyr mimics **68** - **80**.[68]

A recent article by Auclair and co-workers,[69] on the synthesis of inhibitors of aminoglycoside 6'-*N*-acetyltransferases, noted that the interactions between the coenzyme A (CoA) pyrophosphate and the enzyme contained mainly hydrogen bonds. This led to the synthesis of two keto- carboxylates **81** and **82** (**fig. 1.24**), varying the size of linker, as basic pyrophosphate mimics. Analogue **81** bearing the methylene linker showed activity over the ethylene linked analogue **82** in this case.

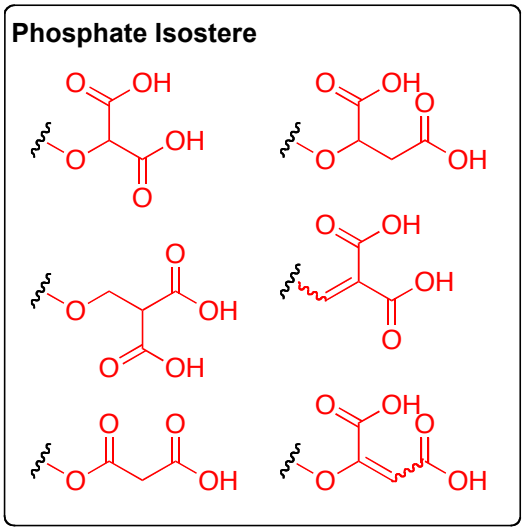


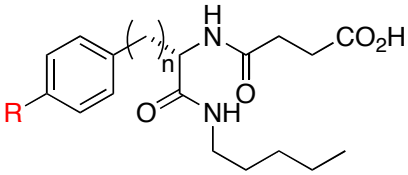


	R
81	CH_2
82	CH_2CH_2

Figure 1.24. Carboxylate-based phosphate mimics **81**, **82**.[69]

In addition to the phosphorus base pTyr mimics, described above (by Larsen *et al.*),[62] they also included a varied range of carboxylate based phosphate isosteres. Here they examined many of the common carboxylate isosteres such as the methylene carboxylate. However, they also included a number of dicarboxylic acid bearing pTyr derivatives. They included the malonate **83** as well as a malonate containing a CH_2 spacer **84** and a malonic ester **85** (**fig. 1.25**). They also varied the position of the CH_2 spacer to include the dicarboxylate **86** and included unsaturated dicarboxylates in the form of **87** and **88** (**fig. 1.25**).





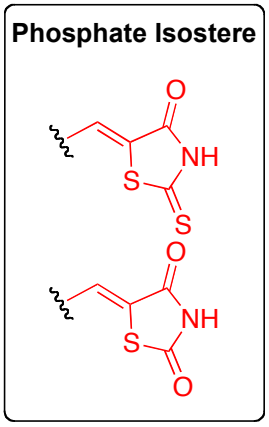
	R
83	$OCH(CO_2H)_2$
84	$OCH_2CH(CO_2H)_2$
85	$OC(O)CH_2CO_2H$
86	$OCH(CO_2H)CH_2CO_2H$
87	$CHC(CO_2H)_2$
88	$OC(CO_2H)CHCO_2H$

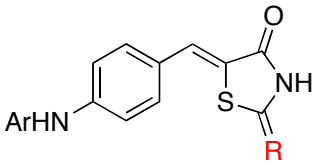
Figure 1.25. Carboxylate-based pTyr mimics **83** – **88**.[62]

1.9. Heterocyclic-based Isosteres

As work continues in pursuit of phosphate isosteres with higher bioavailability, more and more analogues are appearing that bear a heterocyclic scaffold.

A recent publication by Mašič and colleagues details the synthesis and biological evalutation of glutamic acid-based inhibitors of MurD ligase.[70] Mur ligases catalyse the biosynthesis of important peptides for the cell-wall of bacteria. Docking studies of their lead compounds highlighted that its quinazoline ring was located in the same position as the diphosphate of UDP. This prompted them to replace the quinazoline ring with a 2-thioxo-1,3-thiazolidin-4-one (rhodanine) moiety **89, 90** to act as a phosphate isostere (**fig. 1.26**). This had the desired effect giving then their most potent inhibitors to date. Docking studies of their new lead compound confirmed the rhodanine moiety binding in the same location as the diphosphate of UDP.





	R
89	S
90	O

Figure 1.26. Rhodanine-based phosphate mimics **89** and **90**.[70]

A number of pharmaceutical companies have independently converged on the thiadiazolidinone based moieties, that act as pTyr mimics.[40] The fact that they have all converged upon closely related structures speaks for the efficacy of the group as a phosphate isostere. Novartis,[71] AstraZeneca,[72] and Incyte[73] have all developed pTyr mimics containing this group as a phosphate isostere, which all exhibit low μM IC_{50}s for PTP1B (**fig. 1.27**).

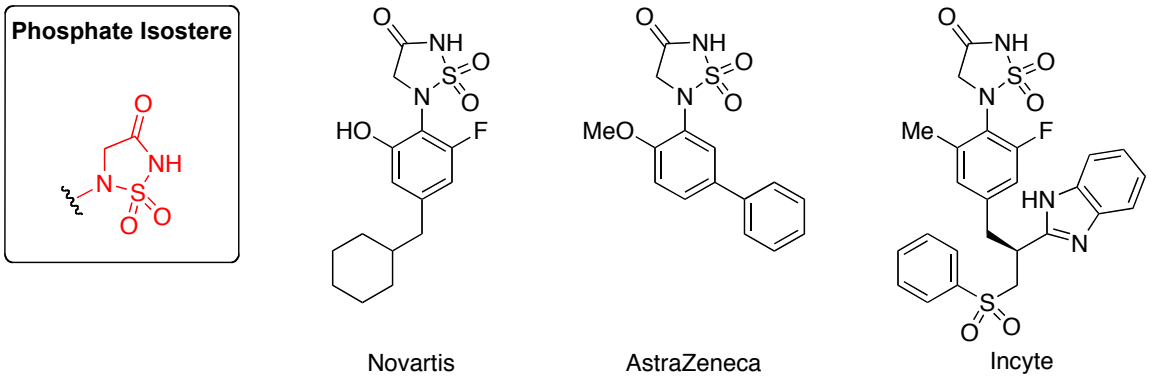
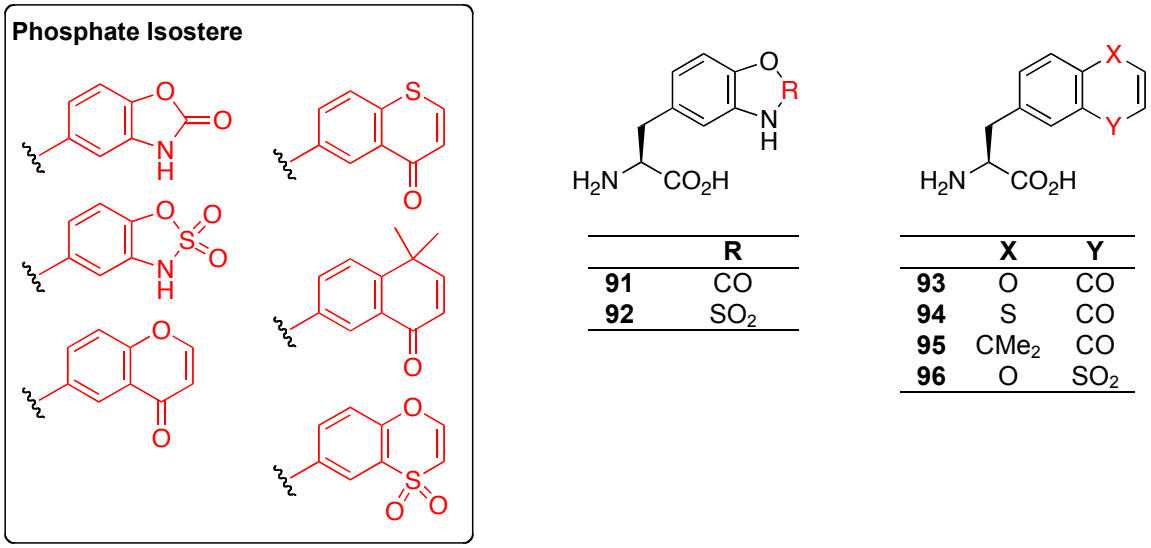


Figure 1.27. Thiadiazolidinone-based phosphate mimics.[40]

Some preliminary work by Blaskovich *et al.*,[74] incorporated several bicyclic moieties **91**-**96** (**fig. 1.28**) into a template structure and screened them against four phosphatases. Some derivatives demonstrated moderate potency and selectivity, indicating the potential utility of these derivatives as non-charged phosphate isosteres with increased bioavailability.



	R
91	CO
92	SO_2

	X	Y
93	O	CO
94	S	CO
95	CMe_2	CO
96	O	SO_2

Figure 1.28. Bicyclic-based phosphate mimics.[74]

1.10. Miscellaneous Phosphate Isosters

A very recent publication by Vasseur and co-workers,[75] reports the synthesis of borono-analogues of all four nucleotide monophosphates **97**-**100** (**fig. 1.29**); the boronic acid in these structures, is acting as the isosteric phosphate

replacement. They conducted semi-empirical calculations that indicated their new bioisosteres are close mimics of their natural counterparts and indicated the utility of this technology in the synthesis of unnatural DNA and RNA.

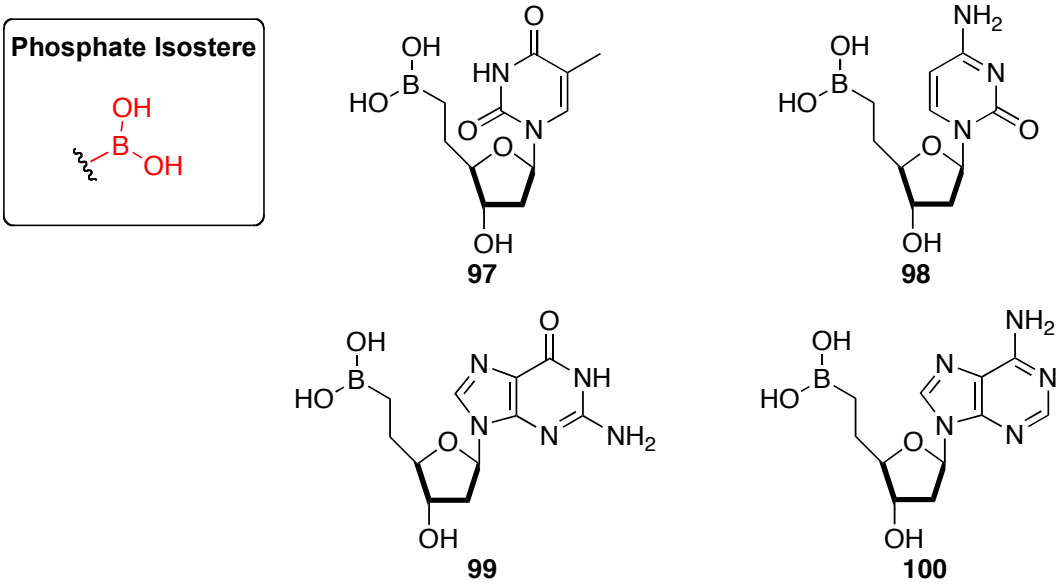


Figure 1.29. All four nucleotides bearing a boronic acid replacement of the phosphate. Borononucleotide T (**97**), borononucleotide C (**98**), borononucleotide G (**99**) and borononucleotide A (**100**).[75]

A commonly overlooked element, in the design of novel phosphate isosteres, due its high toxicity and often unstable nature, is selenium. However, Knapp and co-workers synthesised analogue **101** (**fig. 1.30**) with the seleninate acting as a phosphate replacement in pTyr,[76] and showed that it acted as an irreversible inhibitor of PTPs. An X-ray crystal structure of PTP1B complexed with **101** was obtained, showing that **101** was covalently attached to PTP1B via a selenosulfide bond between Cys215 and the selenium atom.

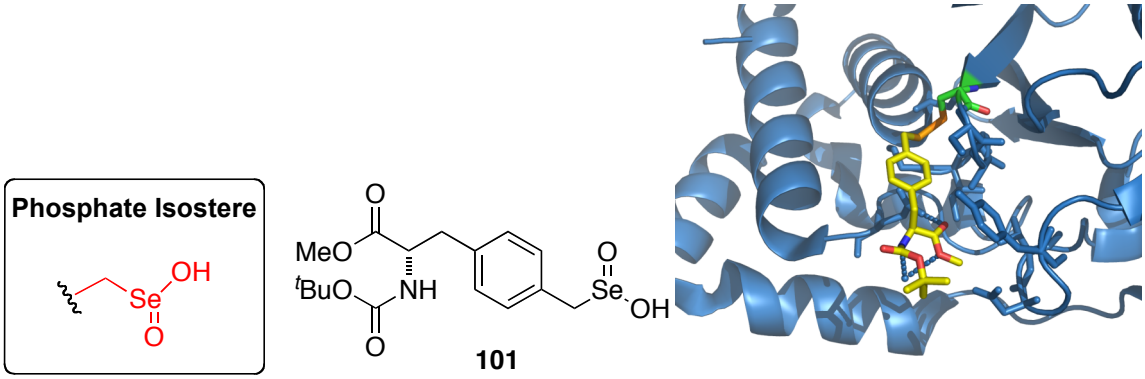


Figure 1.30. Seleninate **101** acting as an irreversible pTyr mimic and the X-ray crystal structure, showing S-Se bond between **101** (Se=light orange, C=yellow, O=red, N=blue) and Cys215 (S=orange, C=green, O=red, N=blue).[76]

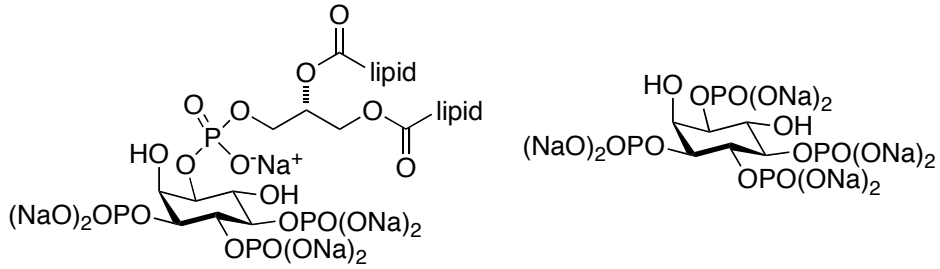
1.11. Summary

The reversible phosphorylation of proteins to transmit signalling information is recognised as one of the most ubiquitous and fundamental processes within the cell. Due to its prevalence within so many cellular processes, the recognition of receptors for phosphorylated cellular moieties has become the target for many small molecule probes. However, due to the highly charged nature of the phosphate and its propensity for hydrolysis by *in vivo* phosphatases it is becoming a less desirable group to incorporate into exogenous ligands. It has therefore become important to develop isosteric groups to replace the phosphate, inorder to increase the ligands metabolic stability and improve its bioavailability. This review has detailed some of the structures that have been incorporated into small molecules as a replacement of the phosphate moiety. The diversity of ligand and targets, means that the phosphate isosteres have been ordered based on broad structural characteristics. It is apparent that there are three main approaches used towards phosphate replacements, these are to base isosteres around phosphorus, sulfur and carboxylic acids. However, there is an increasing shift towards hetrocyclic moieties, these compounds usually have improved pharmaco-kinetic properties, which is important for therapeutic applications. However, highly novel approaches have been shown, that incorporate less common elements such as boron and selenium.

2. Introduction Part 2: PH Domain Targeted Ligand Design

2.1. Introduction and Aims

The primary aim of this project is the synthesis of PtdIns(3,4,5)P_3 **19** analogues. For both chemical and biological reasons, these analogues will be based on the structure of the PtdIns(3,4,5)P_3 head group, Ins(1,3,4,5)P_4 **23** (**fig. 2.1**). Although the lipid diester is undoubtedly important for PBK PH domain binding, and essential for routing PtdIns(3,4,5)P_3 to the cell membrane *in vivo*, *in vitro* Ins(1,3,4,5)P_4 binds to the PKB PH domain with an equivalent affinity.[37] So for the purposes of assessing the contribution each phosphate of PtdIns(3,4,5)P_3 makes for PH domain recognition and binding, omitting the glycerol lipid diester unit is justified.

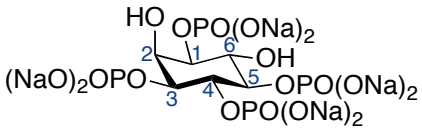


PtdIns(3,4,5)P_3 (19) **InsP_4 (23)**

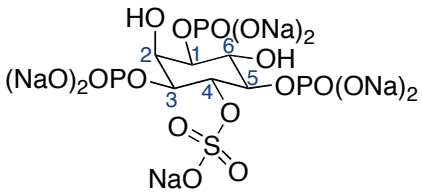
Figure 2.1. The structure of PtdIns(3,4,5)P_3 and its head group InsP_4.

The publication of the X-ray crystal structures of PBKαPH-apo and PBKαPH-Ins(1,3,4,5)P_4 complex by van Aalten and co-workers, highlighted significant differences in the conformation of the PH domain when in the presence and absence of the ligand.[38] Inspection of the ligand-binding site of PBKαPH-apo shows a complex hydrogen-bonding network located around key amino acid residues; Arg86, Lys14, Glu17 and Asn53, and several conserved water molecules (fig. 2.2). Upon binding of Ins(1,3,4,5)P_4, the acidic residue, Glu17, is repelled by the incoming acidic phosphates; it is postulated that this movement enables Arg86 to move 2.3 Å to bind strongly with the 4-position phosphate. Lys14 and Asn53 bind to the 3- and 4-position phosphates, Lys14 moves 1.2 Å

and Asn53 is in a similar position in both apo and complexed states. In addition to the structures of PBKαPH-apo and PBKαPH-Ins(1,3,4,5)P_4 complex, an X-ray crystal structure of PBKαPH-sulfate complex was obtained. The PBKαPH-sulfate complex shows the sulfate located 2.2 Å from the position of the 4-position phosphate in the Ins(1,3,4,5)P_4 complex. The sulfate interacts in a comparable way to the 4-position phosphate binding to both Arg86 and Asn54. It is apparent from our previous work that modification of the 4-position phosphate is not well tolerated for PBKαPH recognition.[31] It was surprising, therefore, that compound **28** (**fig. 2.2**) showed no binding affinity for PBKαPH given the sulfates hydrogen bonding ability. An explanation for this lack of binding maybe that the reduced charge cannot repel Glu17 enough to allow Arg86 to move and form the binding interactions to the conformationally restricted sulfate at the 4-positon.



InsP_4 (23)



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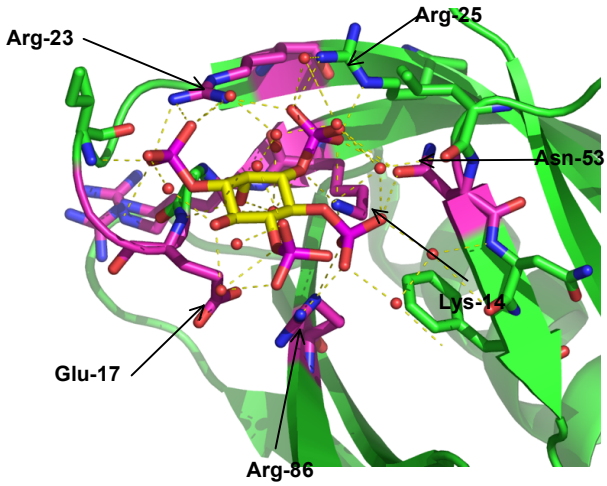


Figure 2.2. 4-Position modified InsP_4 sulfate derivative **28**,[31] and x-ray crystal structure of PBKαPH-Ins(1,3,4,5)P_4 complex,[38] with important residues highlighted.

The 3-position phosphate of Ins(1,3,4,5)P_4 shows hydrogen bonding to Asn53, Lys14 and a single hydrogen bond to Arg23. A significant movement of Arg23 is observed upon binding of Ins(1,3,4,5)P_4 moving 6.2 Å to form a hydrogen bond between the 1- and 3-position phosphates. It can be postulated that an isosteric phosphate replacement of the 3-position phosphate bearing a reduced charge will reduce the interactions observed at this position, potentially with the effect of

preventing the observed conformational change. The synthesis of a small molecule that binds selectively to PKB PH that prevents the conformational change, which appears crucial for PKB activity, and that potentially prevents membrane translocation of PKB will likely be a useful PKB ligand.

It is, therefore, desirable to synthesise analogues of Ins(1,3,4,5)P_4 modified at the 3-position with a phosphate mimic that occupies a similar spatial arrangement as a phosphate but possesses diminished hydrogen bonding, or salt-bridge forming, capability. A moiety such as the sulfate, for example, contains multiple hydrogen bond acceptors, which is akin to a phosphate, but will only be mono charged at physiological pH, reducing its ionic strength. Following on from this, it would be interesting to incorporate the sulfamate moiety, this again possesses good hydrogen-bonding ability, but unlike the sulfate, it will be neutral at physiological pH providing hydrogen bond donor activity as a result. Of additional interest will be the inclusion of the methyl phosphonate moiety, this group will not only make the group mono-charged, but it will also reduce the pK_a of the group with respect to that of the native phosphate. It will therefore be of valuable comparison to synthesise the difluoromethyl phosphonate, as this will have a pK_a closer to that of the native phosphate. Additionally, the 3-position phosphate makes a hydrogen bond with a conserved water molecule within a polar pocket below Arg23 and next to Lys14. Therefore, of interest would be the synthesis of an extended phosphate mimic such as a methylene phosphonate or methylene carboxylate. These, one carbon unit, extended derivatives might favourably displace the water molecule resulting in increased affinity.

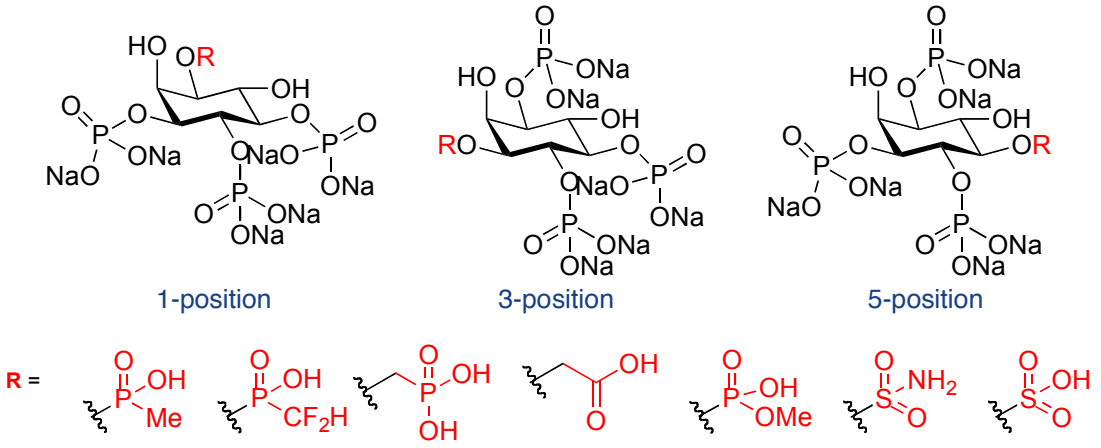


Figure 2.3. Primary synthetic targets, showing the phosphate isosteres that will provide interesting comparisons.

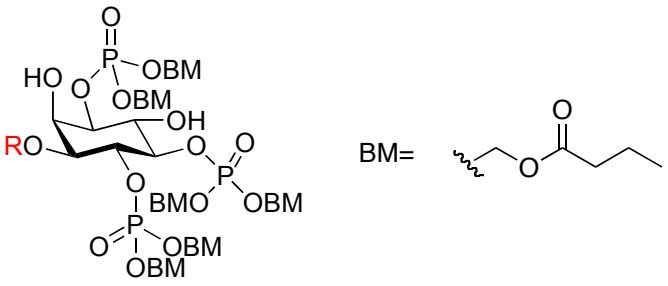
In addition to the synthesis of 3-position modified Ins(1,3,4,5)P_4 derivatives, it will be interesting to extend the group of compounds modified at the 1-position, as the modifications at this position appears to be better tolerated for the GRP1 PH domain than for the PKB PH domain. Of particular interest would be the synthesis of a methyl phosphate ester (**fig. 2.3**) 1-position analogue, as this is more similar to PtdIns(3,4,5)P_3 than any of the previous 1-position modified Ins(1,3,4,5)P_4 derivatives. It is, therefore, expected that this compound might show high affinity for the PKB-PH as well as be tolerated in the GRP1-PH domain. A 1-position methyl phosphate ester analogue probably would not prevent the conformational change in PKB required for activity but instead would likely serve as an inhibitor by preventing membrane translocation.

Synthesis of a range of 5-position-modified Ins(1,3,4,5)P_4 derivatives (**fig. 2.3**) will complete mono-substitution of all the phosphate groups surrounding the inositol ring of Ins(1,3,4,5)P_4. With testing completed for all 1-, 3-, 4- and 5-position analogues we will be able to build a picture of how important each phosphate is for binding to specific PH domains. Knowing the positions where modification is well tolerated, we can then synthesise further PH domain targeted analogues hopefully improving the binding strength and selectivity.

Another potential advantage for omitting the phospholipid portion is for co-crystallisation of our analogues within the PH-domain of PKB or GRP1. Crystallisation of a PBKαPH-PtdIns(3,4,5)P_3 complex or indeed a GRP1-

PtdIns(3,4,5)P_3 complex have been unsuccessful, this has been attributed to the presence of the lipid diester, which is not thought to be essential for binding.

The above set of compounds will initially be tested to ascertain whether they express any binding affinity for the different PH domains. Once a potential inhibitor is found cell membrane permeant groups such as **102** (**fig. 2.4**), successfully employed for the synthesis of cell permeant Ins(1,4,5)P_3 by Holmes *et al.*,[77] could be introduced to enable *in vivo* studies of these compounds.



102

Figure 2.4. Potential cell pembrane permeant $InsP_4$ derivative **102**.[77]

3. Results and Discussion Part 1: Towards 3-position modified PtdIns(3,4,5)P_3 analogues

3.1. Synthetic Targets

The first target is the synthesis of 3-position-modified Ins(1,3,4,5)P_4 derivatives (**fig 3.1**). It is essential that a robust synthesis be developed, as once the initial group of compounds have their biological activity evaluated, it will be necessary to expand the number of derivatives, based on the structure activity relationship that is observed.

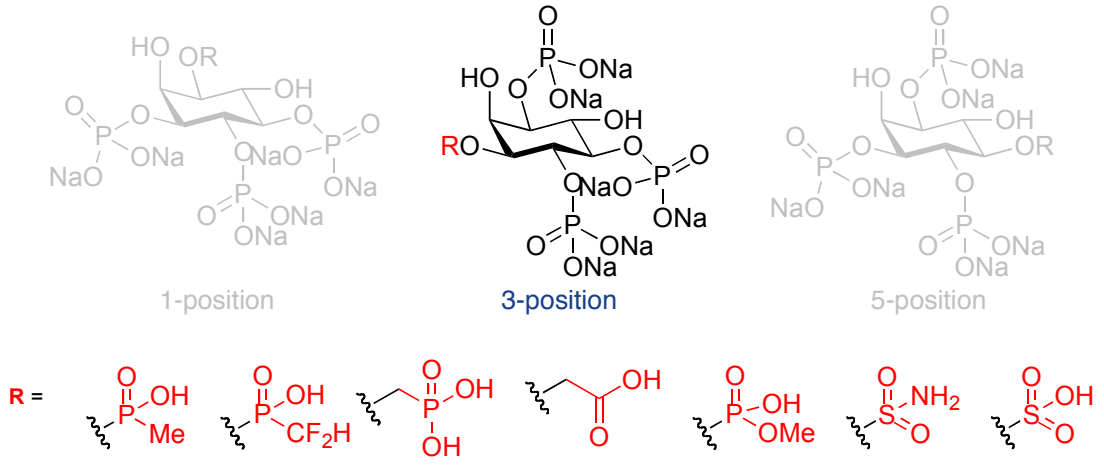


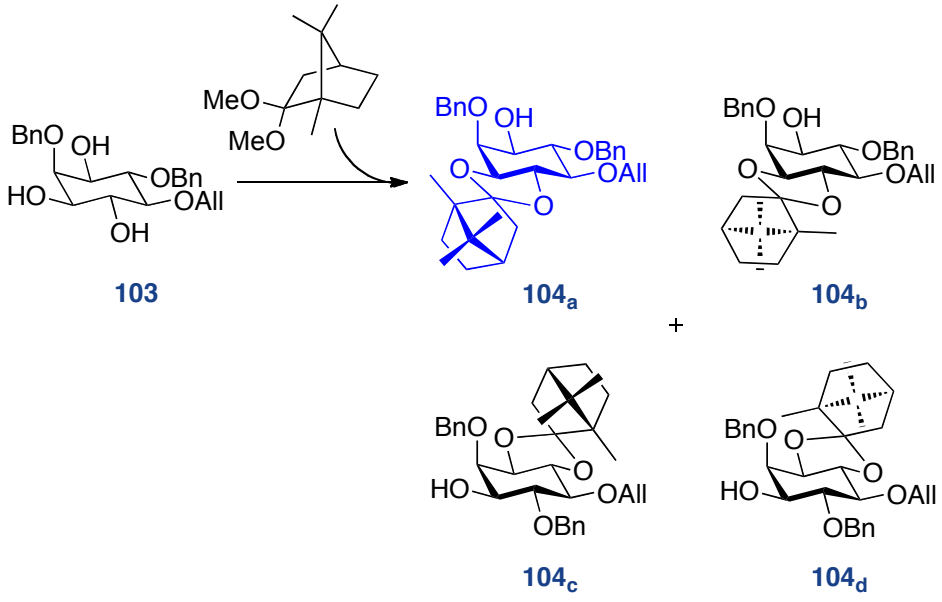
Figure 3.1. 3-Position modified targets.

3.2. Retrosynthesis

Syntheses of inositol phosphates have been approached in a number of different ways.[78,79] The most common approaches use *myo*-inositol as the starting point in their syntheses. *myo*-Inositol is a cheap abundant starting material and benefits from having all the stereogenic centres around the ring correctly defined. The major hurdle to overcome when using *myo*-inositol,[80] is that *myo*-inositol is a *meso* compound and therefore needs to be desymmetrised in order to synthesise enantiomerically pure inositol phosphates, such as our targets. The desymmetrisation of *myo*-inositol has been achieved in a number of different ways, including asymmetric chiral catalysis,[79,81-86] enzyme mediated

desymmetrisation,[87,88] and the use of chiral auxiliaries.[89,90] The use of chiral auxiliaries is the most widely employed technique for the desymmetrisation of *myo*-inositol and is the principle method that is used within our research group.

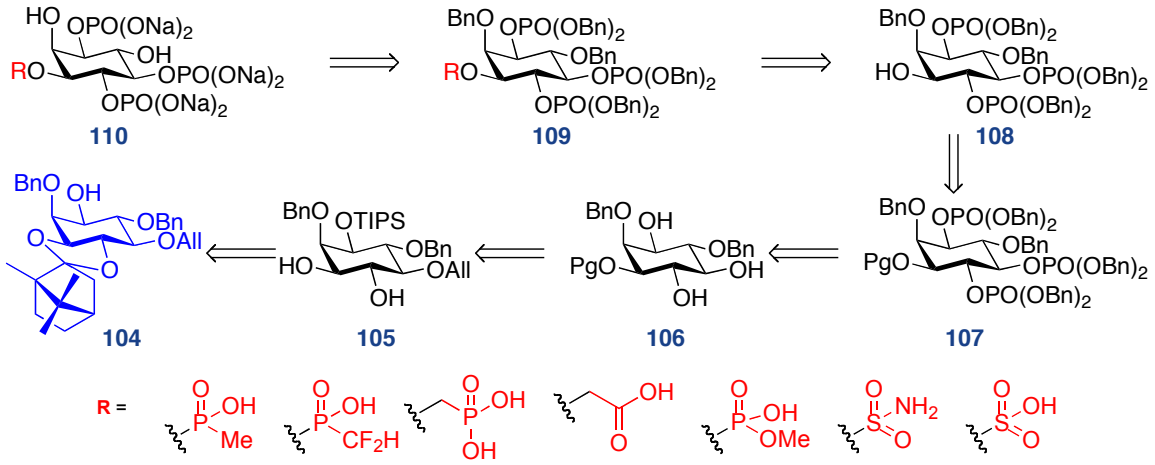
With the chemistry already well established and heavily used within the group, it was decided that the approach towards the synthesis of 3-position Ins(1,3,4,5)P_4 derivatives would centre around the desymmetrisation of *myo*-inositol originally published by Holmes.[89] This desymmetrisation provides a robust route to racemic triol **103**, represented in **scheme 3.1**, and then employs the use of (*S*)-camphor as the chiral auxiliary forming four diastereomers **104** (**scheme 3.1**). **104$_a$** can then be isolated by column chromatography to yield it enantiomerically pure. The use of compound **104$_a$** has already been shown in the development of enantiomerically pure 1-position modified Ins(1,3,4,5)P_4 derivatives.[31] It is hoped that using a similar approach, compound **104$_a$** will deliver robust routes to enantiomerically pure 3-, and 5-position Ins(1,3,4,5)P_4 derivatives.



Scheme 3.1. Diastereomeric resolution of *myo*-inositol derivatives.[89]

A general retrosynthesis for compound **110** for 3-position modified Ins(1,3,4,5)P_4 derivatives is presented in **scheme 3.2**. It is desirable to achieve the requisite

compounds **110** *via* a global de-protection of fully benzyl protected precursor compound **109**, as it has been shown that hydrogenolysis of benzyl protected inositol phosphates generally proceed cleanly, in high yield and with no need for subsequent purification.

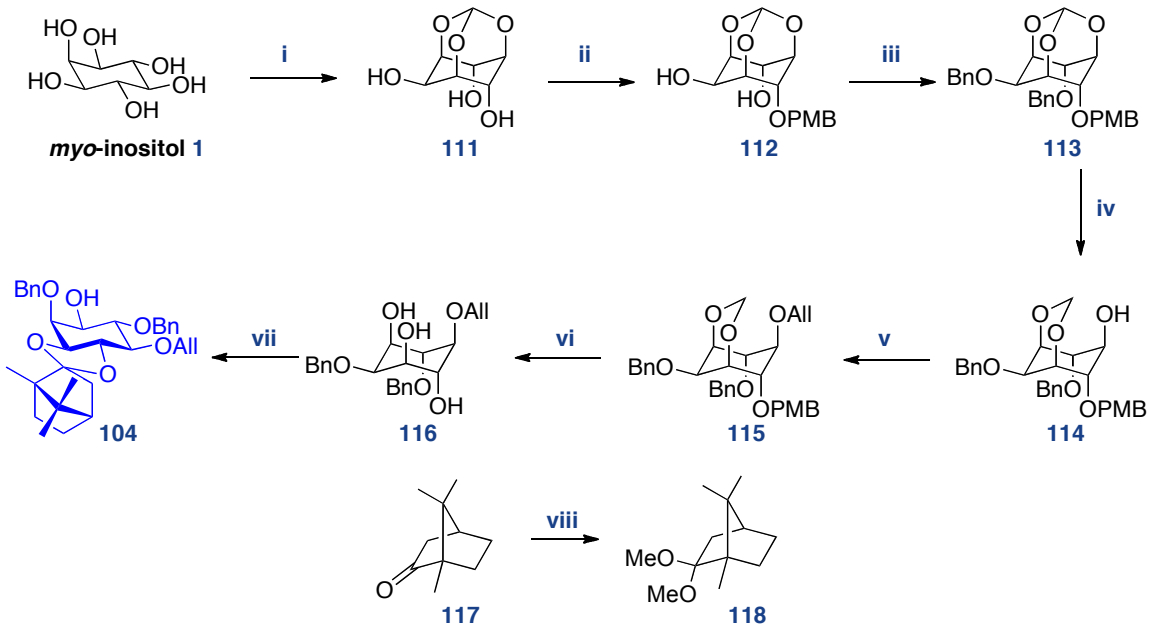


Scheme 3.2. General retrosynthesis for 3-position InsP_4 derivatives.

Additionally, derivatives containing benzyl protected phosphates are amenable to column chromatography and are soluble in a number of common organic solvents. It is then hoped that **109** can be made from the corresponding alcohol **108**. In the forward sense alcohol **108** can potentially undergo multiple derivations for the synthesis of a variety of precursor compounds. Therefore, a reliable synthesis of **108** is certainly a central prerequisite for making a number of 3-position Ins(1,3,4,5)P_4 derivatives. The key alcohol **108** can be obtained by de-protection of a suitable protected trisphosphate **107**. It has been shown that the de-protection of PMB ethers to give their corresponding alcohols in the presence of multiple benzyl protected phosphate esters is well tolerated.[31,91,92] Therefore we proposed the use of PMB-protected trisphosphate **107** which, in turn, could be obtained *via* a phosphitylation reaction from PMB-protected triol **106**. The required 3-position orthogonality is then potentially introduced by regio-selective alkylation, using stannylene acetal chemistry, on the vicinal diol **105** (**scheme 3.2**) which itself can be afforded in 2 steps from enantiopure alcohol **104**.[31]

3.3. Synthesis of alcohol 104

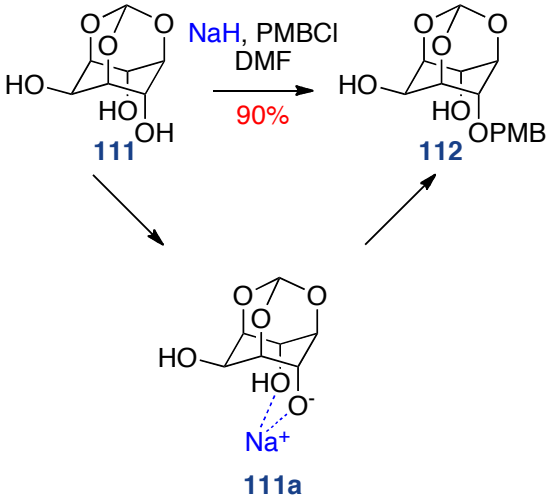
The synthesis of alcohol **104** commences with the protection of *myo*-inositol with triethylorthoformate in the presence of a sub-stoichiometric quantity of 4-toluenesulfonic acid monohydrate (PTSA) giving the adamantane-like inositol orthoformate **111** in 70% yield. It is noteworthy that this reaction is highly consistent and is carried out regularly on scale of up to 50 g.



Scheme 3.3. Holmes synthesis of enantiomerically pure intermediate **104**.[89] *Reagents and conditions*: **i.** $(EtO)_3CH$, $TsOH{\cdot}H_2O$, DMF, 100 °C, 70% yield; **ii.** NaH, PMBCl, DMF, 0 °C → RT, 90% yield; **iii.** NaH, BnBr, DMF, 0 °C → RT, 98% yield; **iv.** DIBAL-H, CH_2Cl_2, 0 °C → RT, 90% yield; **v.** NaH, AllBr, imidazole, DMF, 0 °C → RT, 89% yield; **vi.** HCl, MeOH, reflux, 93% yield; **vii.** (-)-(*S*)-Camphor dimethyl acetal **118**, $TsOH{\cdot}H_2O$, CH_2Cl_2, reflux, 24% yield. Synthesis of Camphor dimethyl acetal **118**. *Reagents and conditions*: **viii.** $HC(OMe)_3$, Montmorrillonite K_{10} clay, hexane, 96% yield.

The triol **111** was mono benzylated regioselectively at a single axial hydroxyl by the careful treatment with sodium hydride and 4-(methoxy)benzyl chloride (PMBCl) to give compound **112** in 90% yield. The high yield and selectivity of this reaction can be attributed to careful formation of the alkoxide. Portion-wise addition of sodium hydride to a solution of triol **111** in dimethylformamide (DMF) kept at -5 ºC ensures mono-deprotonation. The axial alkoxide is favoured over the equatorial partially through a statistical advantage but also through stabilisation of the forming axial alkoxide through chelation of the sodium ion with

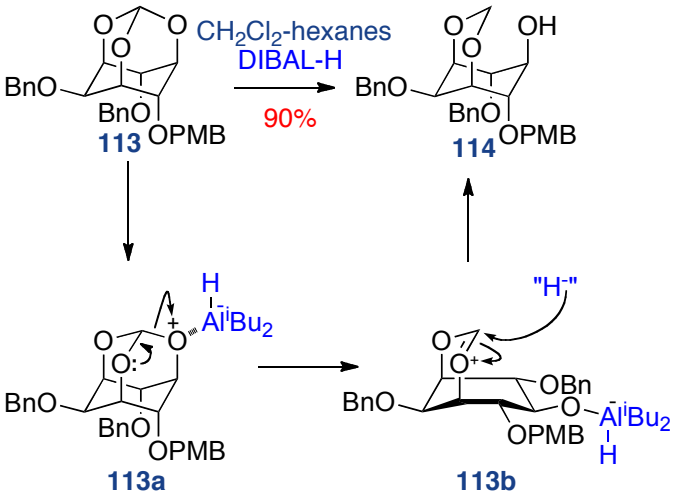
the neighbouring hydroxyl (**scheme 3.4**). Evidence for this chelation effect was provided by Billington *et al.*,[93] who observed that selectivity was lost upon changing either the counter ion or the reaction solvent.



Scheme 3.4. Regio selective PMB ether formation *via* favourable axial alkoxide formation.

The mono-protected PMB ether is not the sole product of this reaction as some of the bis-substituted product is observed, however, these compounds are readily separated by column chromatography. In addition, it should be noted that, although regioselective, the PMB ether formation is not stereoselective, therefore breaking the plane of symmetry of compound **111** in this manner yields a racemic mixture of chiral compounds.

Per benzylation of diol **112** using sodium hydride and benzyl bromide provided the fully protected inositol orthoformate **113** in 98% yield. The orthoformate **113** is reduced by treatment with diisobutylaluminium hydride (DIBALH) in hexanes to exclusively give alcohol **114**. Studies of the reaction mechanism by Holmes *et al.* using deuterium-labelling experiments reveal the reason behind the exceptional selectivity.[94-96] The mechanism is represented in **scheme 3.5**, it shows that at least 2 equivalents of DIBALH are required for the reaction. The first DIBALH equivalent behaves as a Lewis acid coordinating preferentially to the 5-position oxygen of the orthoformate to give **113$_a$**.



Scheme 3.5. Proposed mechanism for the regioselective DIBAL reduction of orthoformate **113.**[96]

The 5-position oxygen is preferred over the 1- and 3-position oxygens most likely due to unfavourable steric interactions with the equatorial benzyl group at the 2-position. This coordination promotes formation of the oxacarbenium ion, which in order to relieve unfavourable 1,3-diaxial interactions flips to give a boat conformation **113$_b$**. With the aluminate species now apart from the oxacarbenium ion, reduction occurs *via* a second equivalent of DIBALH attacking the exposed carbon to give **114**. Compound **114** is formed in excellent yield and without the need for purification by column chromatography.

Alcohol **114** was treated with sodium hydride and allyl bromide to give the fully protected inositol **115**. One-pot methanolysis of both the methylene acetal and the PMB ether of compound **115**, using conc. HCl in methanol, gave the racemic triol **116** in 93% yield.

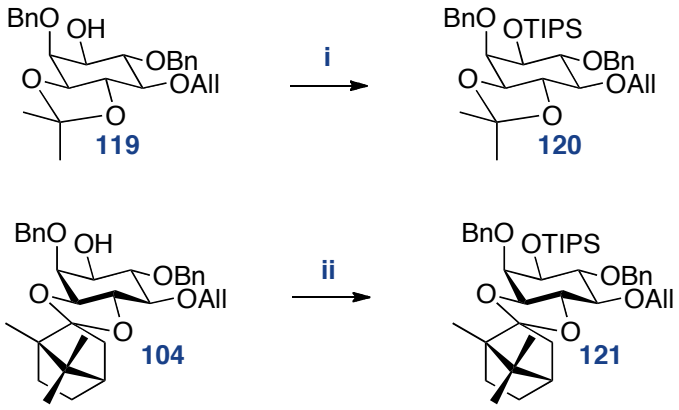
Here we performed the diastereomeric resolution making the synthesis enantiomerically pure. The racemic triol **116** was reacted with the dimethyl acetal of (1*S*)-(-)-camphor **118**, to protect the vicinal diol as the camphor acetal. (1*S*)-(-)-Camphor dimethyl acetal **118** was prepared in one step from (1*S*)-(-)-camphor **117** by reaction with trimethyl orthoformate and Montmorillonite® K-10 clay. The crude reaction mixture gave an 87% conversion to the dimethyl acetal as shown by ^{1}H NMR analysis, the reaction mixture was filtered and concentrated and no further purification was required. The crude dimethyl acetal

118 was reacted directly with triol **116** in the presence of 4-toluenesulfonic acid giving a presumed statistical mixture of the four possible diastereomers of compound **104** (**scheme 3.1**).

With diastereomer **104_a** being slightly more polar than the other diastereomers, it is possible to isolate this compound using column chromatography furnishing the versatile, enantiomerically enriched, and orthogonally protected *myo*-inositol **104** in 24% yield. The other three diastereomers are inseparable and likely accounts for the remaining material.

3.4. Synthesis of alcohol 122

Having made enantiomerically pure alcohol **104**, synthesis of the triisopropylsilyl (TIPS) ether **121** followed by methanolysis could be achieved following the reported synthesis of diol **121** (**scheme 3.6**).[31]



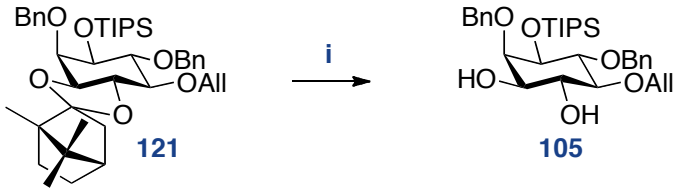
Scheme 3.6. Silyl protection of 1-position hydroxyl.[31] *Reagents and conditions*: **i.** TIPSOTf, 2,6-lutidine, CH_2Cl_2, RT, 94% yield; **ii.** TIPSOTF, Et_3N, CH_2Cl_2, RT, 87% yield.

Entry	Conditions	Yield of 121
1	2,6-lutidine, TIPSOTf, CH_2Cl_2, RT	Reaction did not go to completion
2	Et_3N, TIPSOTf, CH_2Cl_2, RT	Reaction did not go to completion
3	NaH, TIPSCl, DMF, 0 ºC to RT	51%
4[a]	Et_3N, TIPSOTf, CH_2Cl_2, RT	87%

Table 3.1. Summary of reaction conditions for the silyl protection of the 1-position hydroxyl of compound **104**. [a]Et_3N and TIPSOTf pre-stirred for 1 h prior to the addition of alcohol **104**.

The reaction of alcohol **104** in the presence of 2,6-lutidine and triisopropylsilyl trifluoromethanesulfonate (TIPSOTf) was found to be very sluggish, with considerable starting material present, as shown by TLC analysis, after several days stirring at room temperature. The reaction failed to proceed to completion, despite the addition of further amounts of TIPSOTf and 2,6-lutidine. The same result was observed when changing the base to Et_3N (entry 2, **table 3.1**). With tertiary amines failing to effectively promote the reaction, the alkoxide of alcohol **104** formed by NaH in DMF was reacted with triisopropylsilyl chloride (TIPSCl) to give the desired product in a 51% yield (entry 3, **table 3.1**). It was subsequently found, however, that pre-stirring Et_3N with TIPSOTf for 1 h prior to the addition of alcohol **104** gave complete consumption of starting material within 12 h. A much simpler work-up procedure and easier purification gave an isolated yield of 87% for intermediate **121** (entry 4, **table 3.1**).

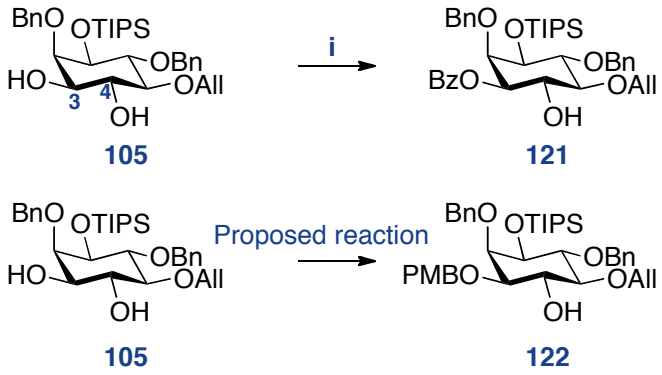
Cleavage of the camphor auxiliary to give the vicinal diol **105** was achieved in 91% yield *via* simple methanolysis using acetyl chloride in methanol and CH_2Cl_2 (**scheme 3.7**).



Scheme 3.7. Cleavage of chiral auxiliary to give vicinal diol **105**. *Reagents and conditions*: **i.** AcCl, MeOH/CH_2Cl_2, RT, 91% yield.

The subsequent synthetic step concerned the installation of an orthogonal protecting group onto the 3-position hydroxyl. A selective protection of this kind was developed within the group where a benzoyl ester functionality was incorporated with exclusive selectivity for the 3-position hydroxyl, over the 4-position hydroxyl, and in 76% yield (**scheme 3.8**).[31,97] This protection involved the formation of a stannylene acetal complex of diol **105**, by reaction with dibutyltin oxide in toluene under reflux, using a Soxhlet apparatus containing 3 Å molecular sieves for the removal of water. Then subsequent reaction of this

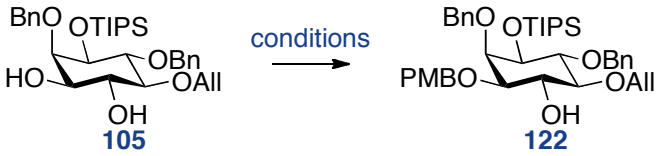
complex with benzoyl chloride (BzCl) at room temperature, gave protection at the 3-position with complete selectivity.



Scheme 3.8. Regioselective benzolyester formation using stannylene acetal chemistry. *Reagents and conditions*: **i.** (a) Bu_2SnO, toluene, reflux; (b) benzoyl chloride, 0 ºC → RT, 80% yield.

The regiochemistry of the benzoylation was shown by X-ray crystallography. It was therefore hoped that an analogous reaction using PMBCl as the electrophile would furnish the desired 3-position PMB ether **122**.

However, using the conditions described, PMBCl showed very little reaction with the stannylene acetal complex of **105** at room temperature after 24 h, showing little consumption of starting material.

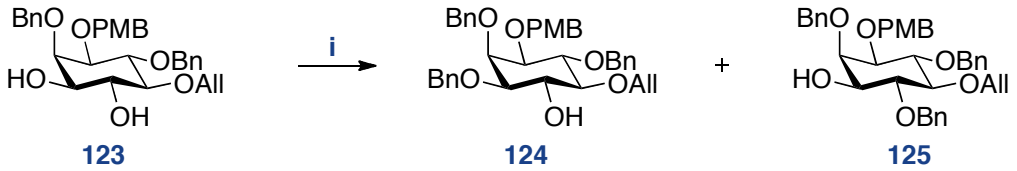


Entry	Conditions	Reaction Time	Yield of 122
1	i. Bu_2SnO, toluene, reflux ii. PMBCl, TBABr, toluene, reflux	+72 h	Complex mixture
2	Bu_2SnO, PMBCl, TBAI, toluene, reflux	18 h	9%
3	Bu_2SnO, PMBCl, TBAI, MeCN, reflux	24 h	33%
4	i. Bu_2SnO, toluene, reflux ii. PMBCl, TBAI, CsF, DMF, 50 ºC	48 h	43%
5	NaH, PMBCl, DMF, 0 ºC to RT	18 h	18%
6	i. Bu_2SnO, toluene, reflux ii. PMBCl, NaI, CsF, DMF, 50 ºC	20 h	Complex mixture
7	i. Bu_2SnO, MeOH, reflux ii. PMBCl, CsF, DMF, 50 ºC	20 h	Complex mixture
8	Bu_2SnO, PMBCl, TBABr, MeCN, reflux	24 h	Complex mixture

Table 3.2. Summary of reaction conditions for the regioselective protection of diol **105** as the PMB ether.

From published literature, it is apparent that reaction of allyl and benzyl halides with stannylene acetals in non-polar media is poor.[97] Frequently, these reactions are conducted in polar solvents such as DMF with much greater success. However, the addition of quarternary ammonium halides has been shown to facilitate a number of reactions of this type in toluene or benzene. Therefore tetrabutylammonium bromide (TBABr) was added to the above reaction conditions but several hours at room temperature failed to give any improvement. Finally, heating the reaction under reflux resulted in a complex mixture of products being formed (entry 1, **table 3.2**). Heating the stannylene acetal under reflux in toluene with PMBCl and tetrabutylammonium iodide (TBAI), on the other hand, yielded 9% of the desired PMB ether with no trace of the undesired 4-

position PMB ether. It is apparent from these two results that PMBCl is much less reactive towards the stannylene than BzCl in non-polar media, despite the addition of quarternary ammonium halides. Hence, further study of the reaction was required, the results of which are summarised in **table 3.2**. Conditions developed within the group selectively benzylate the 3-position of a comparable protected *myo*-inositol **123** (**scheme 3.9**).[91] This reaction involves heating a solution of dibutyltin oxide (1.1 equivalents), benzyl bromide (4.8 equivalents), and TBAI (1 equivalent) in acetonitrile under reflux, using a Soxhlet apparatus containing 3 Å molecular sieves to remove the water generated through stannylene acetal formation. The reaction proceeds in 72% yield with a 5:1 selectivity (as ajudged by ^{1}H NMR analysis) in preference for the 3-position benzyl protected compound **124** over the 4-position **125** (**scheme 3.9**).

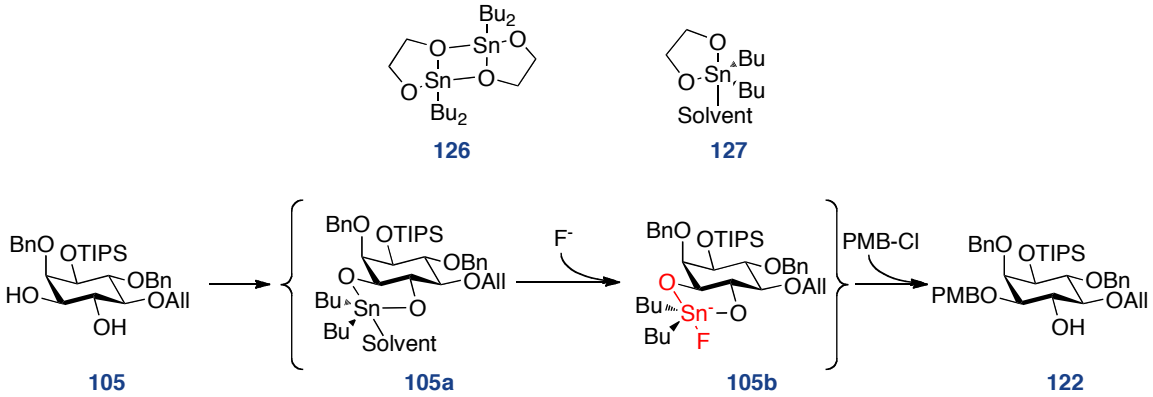


Scheme 3.9. Regioselective benzylation of vicinal diol **123** developed within the group.[91] *Reagents and conditions*: **i.** Bu_2SnO, TBAI, benzyl bromide, acetonitrile, reflux, 72% yield.

The analogous reaction using intermediate **105** instead of compound **123** and PMBCl instead of benzyl bromide indeed gave a marked improvement, giving an isolated yield of 33% for desired compound **122** (entry 3, **table 3.2**). The presence of a polar aprotic solvent clearly facilitates the reaction; this could be attributed to improved stabilisation of the presumed S_N2 transition state. The literature on reactions of this type have described a two-step procedure, initially the relatively stable stannylene acetal is pre-formed, in a non-polar solvent. The second step involves replacement of the non-polar solvent with a polar solvent, in which the stannylene acetal is reacted with the appropriate electrophile. Thus, diol **105** and dibutyltin oxide (1.1 equivalent) in toluene where heated under reflux together with a Soxhlet apparatus attached containing 3 Å molecular sieves. After 12 h, the toluene was removed by distillation to give the stannylene acetal, as a wax, which was then dissolved in dry DMF at room temperature.

The solution of the stannylene acetal was treated with PMBCl at room temperature, but the reaction appeared to progress slowly and heating to 50 ºC showed no improvement in the reaction progress. Only upon the addition TBAI was significant consumption of starting material observed.

Examination of the mechanisms associated with reactions of this type have been discussed in the literature.[97] Using mass spectrometry and ^{119}Sn NMR techniques it has been shown that in non-polar media the stannylene acetal exists as a dimer **126** of the type shown in **scheme 3.10**. Here, the tin atom is five-coordinate, with one of the oxygen atoms tri-coordinate. When all solvent is removed, the dimers are thought to possibly exist as oligomers or infinite ribbons with a six-coordinate tin atom. In polar solvents a trigonal bipyramidal structure **127** can be formed by coordination of one molecule of solvent to the central tin atom (**scheme 3.10**).

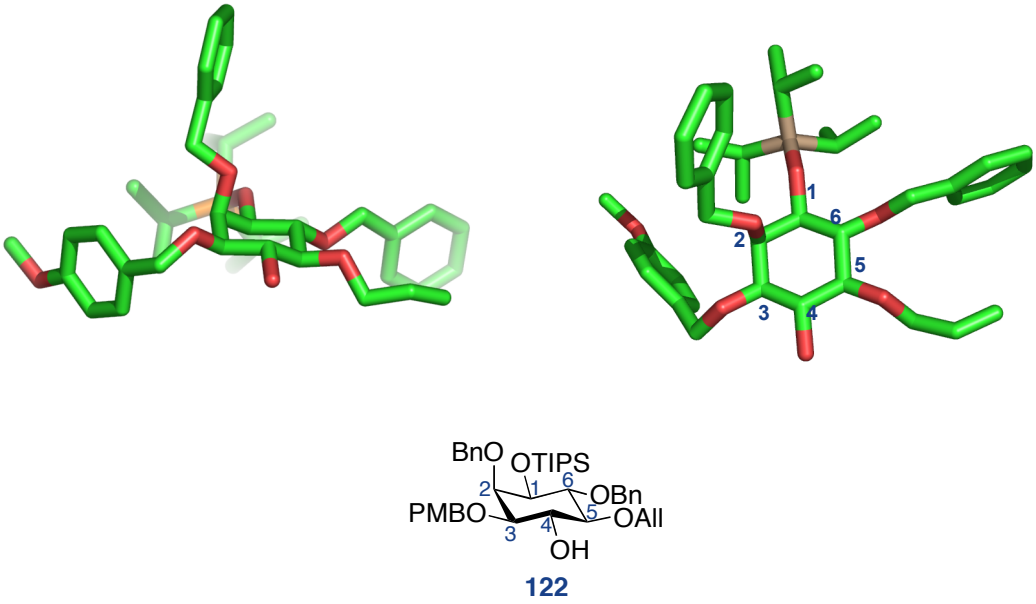


Scheme 3.10. Representation of stannylene acetals in solution.[97]

In these complexes the apical oxygen possesses the longer O-Sn bond so typically has the greater reactivity. A common technique for improving the reactivity of the apical oxygen is to add caesium fluoride to the reaction mixture. The fluoride is able to replace the coordinating solvent (**105b, scheme 3.10**) polarising the apical oxygen thus increasing its reactivity.

For this reason, caesium fluoride was also added to the above reaction, subsequent work-up and isolation *via* column chromatography gave the desired 3-position PMB ether **122** in 43% isolated yield, and a 2:1 ratio, as adjudged by

^{1}H NMR analysis, in favour of the desired 3-position protected product over the 4-position (entry 4, **table 3.2**). The stereochemistry was assigned by 1D and 2D ^{1}H NMR techniques. Subsequently, slow controlled diffusion of petroleum ether, as an anti-solvent, into a concentrated solution of **122** in ethyl acetate yielded a crystal of sufficient quality to obtain X-ray crystal analysis, which confirmed our initial NMR assignment by showing the relative configuration (**fig. 3.2**).



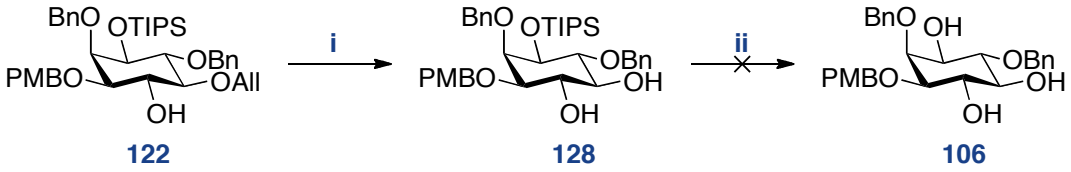
122

Figure 3.2. Single crystal X-ray structure of intermediate **122** confirming desired regiochemistry.

Further attempts to optimise the reaction conditions failed to improve the selectivity or yield (**table 3.2**). Interestingly, substitution of TBAI with NaI severely reduced the reactivity, which eventually led to a complex mixture of products (entry 6, **table 3.2**). This indicates that the quaternary ammonium species plays an important role in the reaction. In addition to this observation, work conducted within the group showed that an improvement in yield was obtained when adding TBABr to their reaction of stannylene acetal with benzyl bromide. In this instance halide exchange cannot produce a more reactive electrophile and therefore the tetrabutyl ammonium species must be the contributing factor for the improvement in reaction. It is postulated, therefore,

that the tetrabutyl ammonium ion is able to improve solubility of the halide ion in the polar organic solvent. This improved solubility allows for increased Sn-halide coordination, which thus increases reaction rates, leading to less degradation of the stannylene, and consequently an improvement in yield.

Following the successful isolation of intermediate **122**, sequential removal of the allyl and TIPS protecting groups to furnish triol **106** was required prior to phosphitylation.



122 **128** **106**

Scheme 3.11. Synthesis of triol **106.** *Reagents and conditions*: **i.** (a) Wilkinson's catalyst, DIPEA, EtOH, reflux, (b) 4-TsOH·H_2O, MeOH, RT, 76%; **ii.** TBAF, THF, RT.

The allyl protecting group was removed by firstly treating **122** with Wilkinson's catalyst, which after 3 h complete isomerisation of the allylic double bond to give the enol ether was shown by ^{1}H NMR analysis. Methanolysis of the enol ether using PTSA gave **128** initially isolated as a crude gum. ^{1}H NMR analysis of this material indicated that the allyl group had been cleaved by the loss of signals corresponding to both the allylic and vinylic protons. However, purification of intermediate **128** proved difficult, requiring exhaustive chromatography isolating **128** in 76% yield (**scheme 3.11**).

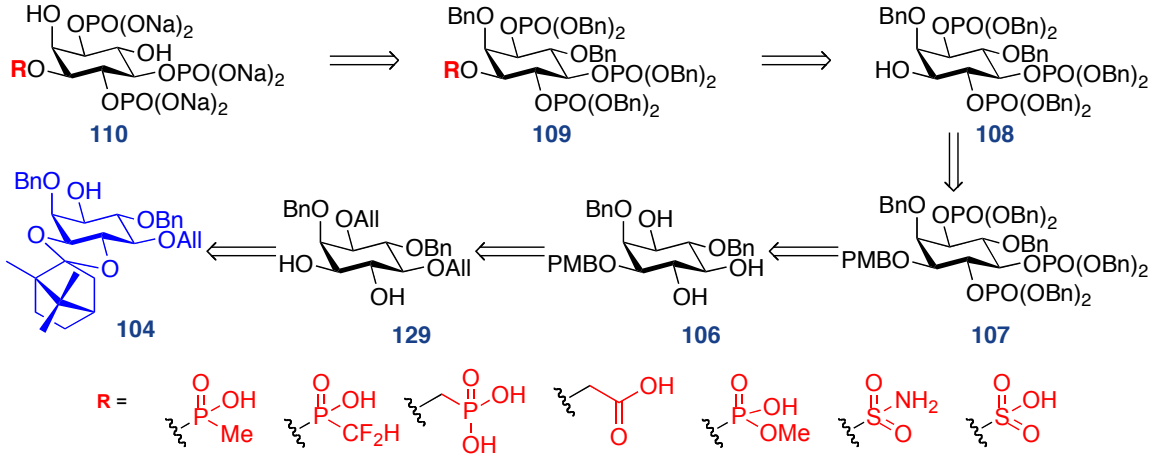
Having isolated intermediate **128**, removal of the TIPS protecting group to furnish triol **106** was attempted using standard silyl deprotection conditions. Thus diol **128** was treated with tetrabutyl ammonium fluoride (TBAF) in THF at room temperature. Consumption of the starting material was observed after stirring for 18 h, correlating with the appearance of a new more polar product as adjudged by TLC analysis. However, work-up followed by isolation *via* column chromatography yielded a very impure brown oil that corresponded to multiple products and only 30% of the required mass. The reaction was repeated with fresh TBAF to rule out the possibility that the initial reagent used might have degraded, causing undesirable side reactions. This yielded a sticky pale yellow

solid, which by ^{1}H NMR suggested was the desired product. However the material showed considerable impurities and also corresponded to only 12% of the desired mass.

Therefore, due to the poor yield and exhaustive purification experienced with the regioselective PMB protection of diol **128** and the subsequent problems experienced with the TIPS deprotection of diol **128**, a new approach towards the synthesis of 3-position modified Ins(1,3,4,5)P_4 derivatives was envisaged.

3.5. Synthesis of alcohol 131

The potential othogonality obtained by the incorporation of the TIPS protecting group at the 1-position hydroxyl is desirable because it adds potential flexibility to the intermediates formed, which could be useful for synthesis of more complex inositol phosphates in the future. However, for the initial target alcohol **108** this orthogonality is not required so a new retrosynthesis can be postulated (**scheme 3.12**).

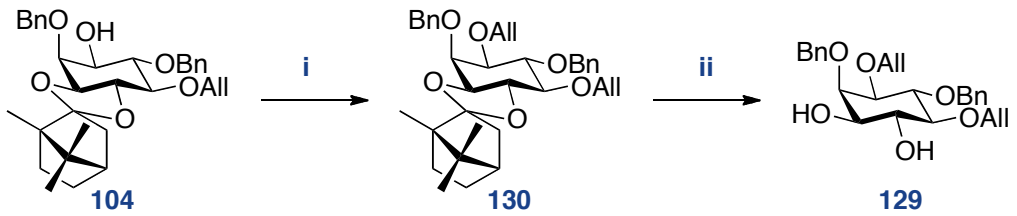


Scheme 3.12. Retrosynthesis of revised synthetic route to 3-position modified Ins(1,3,4,5)P_4 derivatives.

As with the initial route, it is key to achieve orthogonality at the 3-position hydroxyl group. The 1-, 4-, and 5-position hydroxyl groups will ultimately all need to be phosphorylated, it is simplest to do this in one step from the desired triol **106**. Therefore, orthogonality is not required at the 1-, 4- and 5-positions. A

shorter synthesis is proposed by forming the 3-position PMB ether **106** from known diol **129**. With allyl being a considerably smaller group than TIPS it was hoped that the stanylene acetal of **129**, formed during the regioselective alkylation step, might be more reactive towards PMBCl, because of the reduced steric bulk at the 1-position, leading to improved protection of the 3-position alcohol.

In the first instance, synthesis of diol **129** was accomplished inaccordance with the literature procedure (**scheme 3.13**).[91,92] Thus **104** was treated with NaH and allyl bromide in a mixture of THF and DMF with a sub-stoichiometric quantity of imidazole, giving the desired bis allyl protected intermediate **130** in 68% yield. The camphor acetal was cleaved in 95% yield by treatment of **130** with acetyl chloride in CH_2Cl_2 and MeOH.

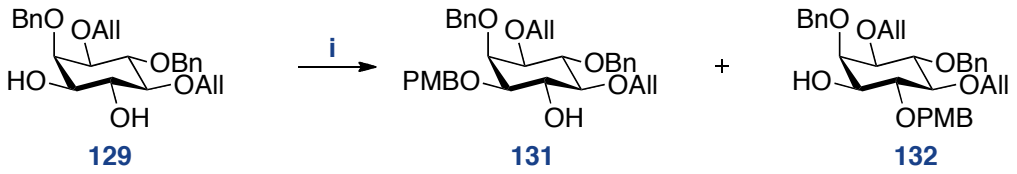


Scheme 3.13. Synthesis of diol **129**. *Reagents and conditions*: **i.** NaH, AllBr, THF, imidazole, 0°C to RT, 68% yield; **ii.** AcCl, CH_2Cl_2, MeOH, RT, 95% yield. Improved synthesis. *Reagents and conditions*: **i.** NaH, AllBr, DMF, TBAI, 0°C to RT, telescoped to following step; **ii.** AcCl, CH_2Cl_2, MeOH, RT, 93% over 2 steps.

A subsequent improvement to the synthesis of diol **129** was found by simply adding NaH (2 eq), allyl bromide (2 eq) and TBAI (cat) to a cooled solution of alcohol **104** in DMF. The reaction was allowed to warm to room temperature overnight, aqueous work up gave the crude product that was dissolved in CH_2Cl_2 and MeOH and treated with acetyl chloride to effect methanolysis of the camphor acetal. Isolation of the desired diol **129** was achieved by column chromatography giving the product in 93% yield over the two steps (**scheme 3.13**).

Having isolated diol **129** in excellent yield, it was important to achieve the subsequent regioselective protection of the 3-position hydroxyl in good yield to

provide enough material for the remaining steps of the synthesis. Therefore, using the previously optimised conditions the stannylene acetal of diol **129** was formed by reaction with dibutyltin oxide in refluxing toluene with a Soxhlet apparatus containing 3 Å molecular sieves. After 18 h, the reaction solution was concentrated then re-dissolved in DMF and treated with PMBCl, TBAI and CsF. As the reaction progress was monitored, formation of multiple products were observed on TLC analysis, and ultimately a complex mixture was formed.



Scheme 3.14. Regioselective PMB ether formation from vicinal diol **129**. *Reagents and conditions*: **i.** Bu_2SnO, TBABr, PMBCl, acetonitrile, reflux, 25% yield.

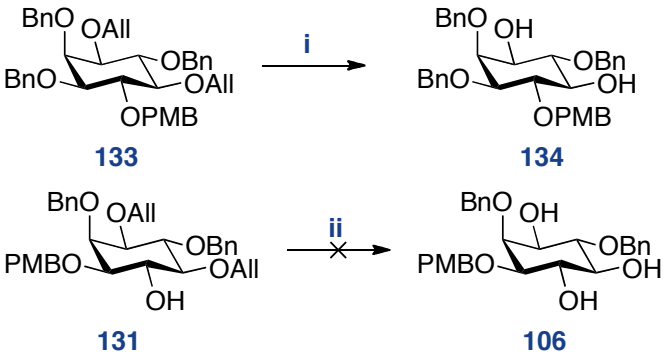
No isolation of products or further analysis was conducted, as a cleaner reaction was found by simply refluxing diol **129** with dibutyltin oxide, PMBCl and TBABr in acetonitrile with a Soxhlet apparatus containing 3 Å molecular sieves. After the work-up procedure, ^{1}H NMR analysis of the crude material indicated that a 5:2 ratio of mono PMB-protected isomers was formed. Separation of the isomers was troublesome, but after multiple chromatographic columns the major isomer was isolated in 25% yield and corresponded to the desired 3-position protected PMB ether **131** as assigned by ^{1}H NMR analysis (**scheme 3.14**).

A higher yield for the PMB ether formation is desirable for a more robust synthesis, however, the reduced number of synthetic steps over the initial route proposed might moderately compensate for the problematic step and still provide a viable synthetic route. So, despite the poor yield of the PMB ether formation, removal of the allylic ethers to give the desired triol was attempted.

The widely used and typically reliable method for removal of an allyl ether is to firstly use Wilkinson's catalyst to isomerise the double bond to the enol ether and then cleave this by acid catalysed methanolysis. This method was successfully employed for the synthesis of intermediate **128** reported above. However, one of the major disadvantages of this method is the partial reduction of the allyl ether to

the propyl ether that occurs, usually only in a small proportion. The propyl ether is not hydrolysable by methanolysis and as **131** contains two allyl ether groups there is a statistically increased chance for reduction taking place and thus potentially limiting the reaction yield. However, a literature method developed by Boons *et al.*,[98] treats Wilkinson's catalyst with *n*-butyl lithium to form a catalyst that isomerises allyl ethers with no discernable reduction to the proply ether. It is thought that the chloride ion in Wilkinson's catalyst is replaced by an *n*-butyl ion that undergoes a β-hydride shift to form the new active catalyst, hydrido tris(triphenylphosphine) rhodium(I).

This modified catalyst was put to good use within the group for the removal of two allyl ethers from a similar system (**scheme 3.15**).[91,92]



Scheme 3.15. Modified Wilkinson's catalyst removal of allylic ethers. *Reagents and conditions*: **i.** (a) Wilkinson's catalyst, BuLi, THF, reflux; (b) AcCl, CH_2Cl_2/MeOH, RT, 89% yield. **ii.** (a) Wilkinson's catalyst, BuLi, THF, reflux; (b) AcCl, CH_2Cl_2/MeOH, RT.

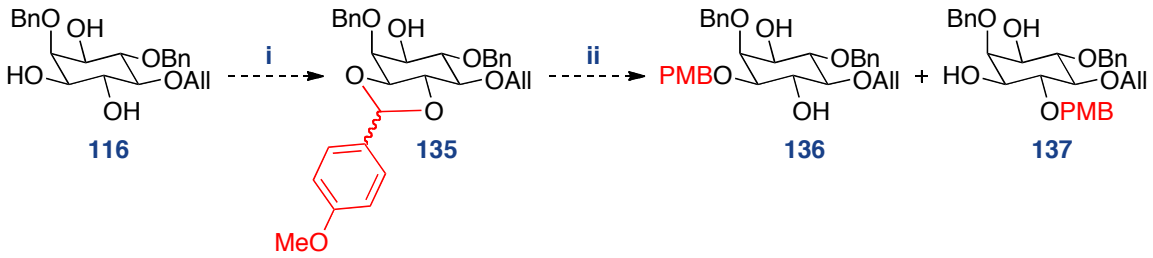
Wilkinson's catalyst was thus treated with *n*-butyl lithium at -78 °C and then allowed to stir at room temperature for 10 minutes, before the addition of bis allyic ether **131**. The reaction was heated under reflux and the isomerisation progress was followed by ^{1}H NMR analysis. However, after 6 h, analysis of the crude reaction mixture showed the allylic ether was still present and that no isomerisation had occurred. The reaction was thus administered with fresh untreated Wilkinson's catalyst, which led to the required isomerisation within 3 h. The crude material was then treated with acetyl chloride in methanol and CH_2Cl_2 to methanolyse the enol ether. Isolation of the product was attempted by silica

gel column chromatography, this gave a sticky blue gum and ^{1}H NMR analysis was inconclusive as to whether the triol had formed.

Given the problematic nature of the selective PMB ether formation and the subsequent loss of material experienced with the removal of the allyic ethers, it was decided that an improvement in the regioselective protection of the 3-position hydroxyl was vital for a workable synthesis of 3-position modified Ins(1,3,4,5)P_4 derivatives.

3.6. Synthesis of Triol 106

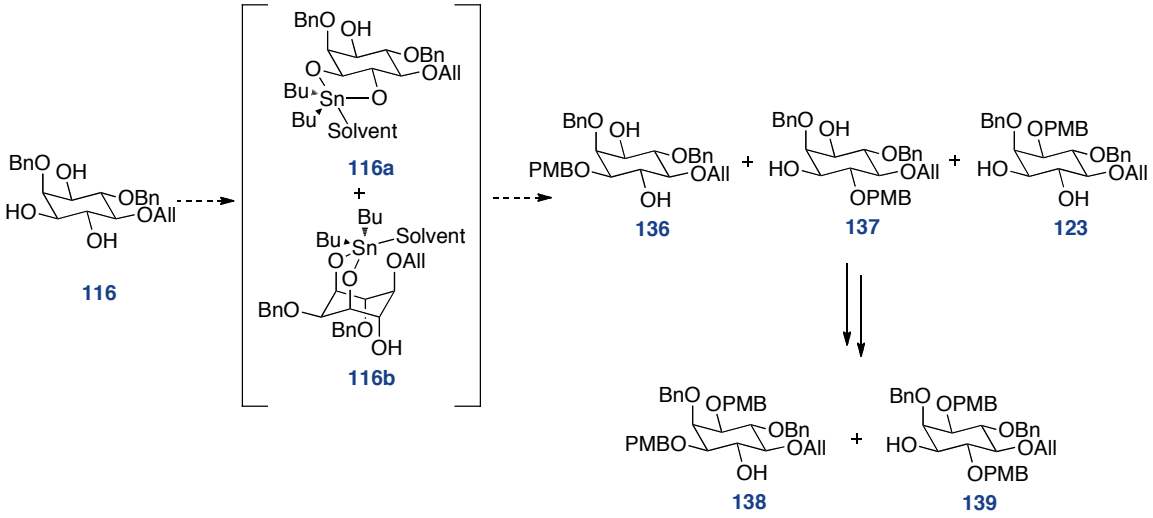
It had so far proved difficult to reliably install the desired PMB ether at the 3-position using stannylene acetal chemistry; it was thought that an improvement might be achieved by using a different orthogonal protecting group. However, two speculative methods were attempted prior to changing the protecting group. Firstly it is widely documented that 1,3-diols are condensed with anisaldehyde dimethyl acetal to give the corresponding anisylidene acetal, which readily undergo reduction with DIBALH to give the corresponding PMB ether, often with excellent selectivity.[99] It was, therefore, hoped that triol **116**, which can be formed in enantiopure form *via* acid hydrolysis of the camphor auxiliary of **104**, might form the 5-membered anisylidene acetal **135** (**scheme 3.16**) which, in turn, might then undergo selective reduction with DIBALH to give the desired 3-position PMB ether. It was speculated that the 3-position PMB ether would be the favoured product based on a favourable steric environment provided by the axial 2-position benzyl ether over the equatorial 5-position allylic ether.



Scheme 3.16. Desired anisylidene acetal formation and reduction. *Reagents and conditions*: **i.** anisaldehyde dimethylacetal, TsOH·H_2O, DMF, 60 °C, 100 mbar pressure; **ii.** DIBAL-H.

A dry solution of racemic triol **116** in DMF was therefore treated with anisaldehyde dimethylacetal and PTSA, and heated to 60 °C under 100 mbar pressure in order to drive off the methanol produced with the new acetal formation. But after 3 days under these conditions, no progression of the reaction was observed; and as a result the reaction was terminated.

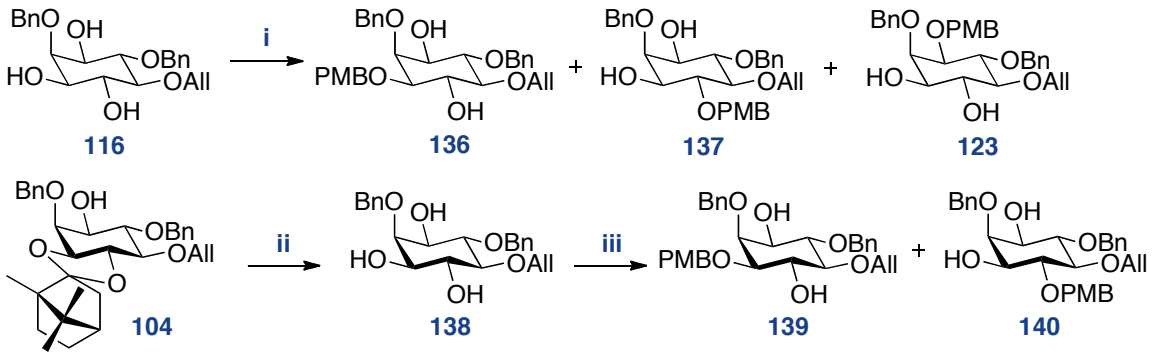
The second speculative reaction was to attempt using the stannylene acetal chemistry on racemic triol **116** (**scheme 3.18**), installing the PMB ether on the enantiopure version of triol **116** would have the benefit of reducing the length of the synthesis. However the presence of the extra hydroxyl group might lead to the possible formation of the six membered stannylene acetal **116$_b$**, potentially leading to further undesired PMB ethers **137, 123, 138** and **139** (**scheme 3.17**).



Scheme 3.17. Possible products for reaction of triol **116** using stannylene acetal chemistry.

Nevertheless, the stannylene acetal of triol **116** was formed by heating under reflux with dibutyltin oxide in toluene for 18 h through a Soxhlet apparatus containing 3 Å molecular sieves. The toluene was removed under reduced pressure yielding a waxy solid, which was dissolved in DMF. PMBCl, TBAI and CsF were added to the solution of stannylene acetal and the reaction heated to 50 °C. This reaction formed two major products, work-up and isolation of these products revealed two mono protected PMB ethers in a 2:1 ratio. The major product was revealed as the desired 3-position PMB ether **136** isolated in 50%

yield and the stereochemistry was assigned by ^{1}H, COSY, HSQC and HMBC NMR analysis (*vide infra*). The minor 4-position PMB ether **137** was isolated in 23%, the remaining mass can be accounted for as unreacted starting material, no other regioisomers were observed (**scheme 3.18**).



Scheme 3.18. Synthesis of racemic and enantiopure 3-position PMB ethers. *Reagents and conditions*: **i.** (a) Bu_2SnO, toluene, reflux, (b)TBAI, PMBCl, CsF, 50°C, 50% yield. Enantiomerically pure synthesis of 3-position PMB ethers. *Reagents and conditions*: **ii.** AcCl, CH_2Cl_2, MeOH, RT, 76%; **iii.** (a) Bu_2SnO, toluene, reflux, (b)TBAI, PMBCl, CsF, 50°C, 50% yield.

The reaction proved reliable and repeatable on a large scale; the separation of the two observed regioisomers was readily achieved with a single silica gel chromatographic column. In order to produce the enantiomerically enriched PMB ether of **138,** a solution of alcohol **104** in CH_2Cl_2 and MeOH was treated with acetyl chloride to effect the methanolysis of the camphor acetal, this gave the enantiomerically pure triol **138** in 76% yield. This reaction gave enantiomerically pure **139** using the conditions described above (**scheme 3.18**).

It was important to correctly assign the regiochemistry observed in formation of compound **139**, this was achieved using a combination of 1D and 2D ^{1}H and ^{13}C NMR techniques. The 2-position proton expresses a very characteristic signal in the ^{1}H NMR spectrum, as it is the only proton that has two equatorial-axial couplings, as a result this proton is easily assigned. Using the 2D ^{1}H-^{1}H COSY the 2-position proton showed two correlations at 3.3 ppm and 3.5 ppm (**fig. 3.3**). The signal at 3.5 ppm showed correlation to an alcohol peak and the signal at 3.3 ppm did not show correlation to an alcohol, this rules out the possibility of the compound being the 4-position PMB ether **140**. The signal at 3.5 ppm also

showed two correlations at 3.7 ppm and 4.0 ppm. The signal at 4.0 ppm corresponds to the already assigned 2-position proton. However, the signal at 3.7 ppm does not show a correlation to an alcohol, this rules out the presence of a vicinal diol and so consequently rules out the possibility of the compound being the 1-position PMB ether **123**. This process of elimination provides strong evidence for the compound being substituted at the 3-position. Using ^{1}H-^{13}C 2D HSQC and HMBC NMR techniques the ^{1}H signal at 3.3 ppm (now firmly assigned as 3-position proton) showed a ^{13}C HSQC correlation at 83 ppm (**fig. 3.4**). The ^{13}C signal at 83 ppm showed a strong HMBC correlation to two ^{1}H signals at 4.55 ppm and 4.65 ppm (**fig. 3.4**), these signals correspond to the AB quartet of the benzylic protons belonging to the PMB ether. This confirms that the PMB ether has formed selectively at the 3-position.

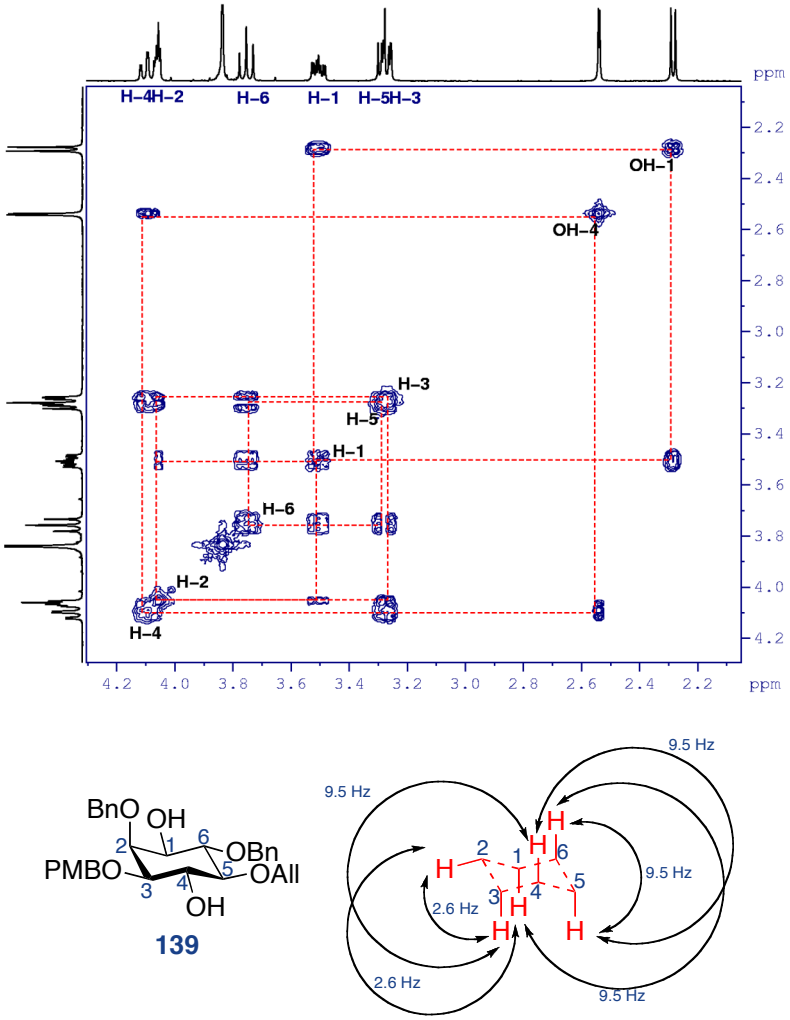


Figure 3.3. ^{1}H-^{1}H NMR correlation spectrum (COSY) of diol **139**, the dashed red lines indicate protons that couple to one another. Structure of **139** and diagram showing the coupling of the ring protons and their coupling constants in Hz (*J* values were obtained from the 1D ^{1}H NMR spectrum).

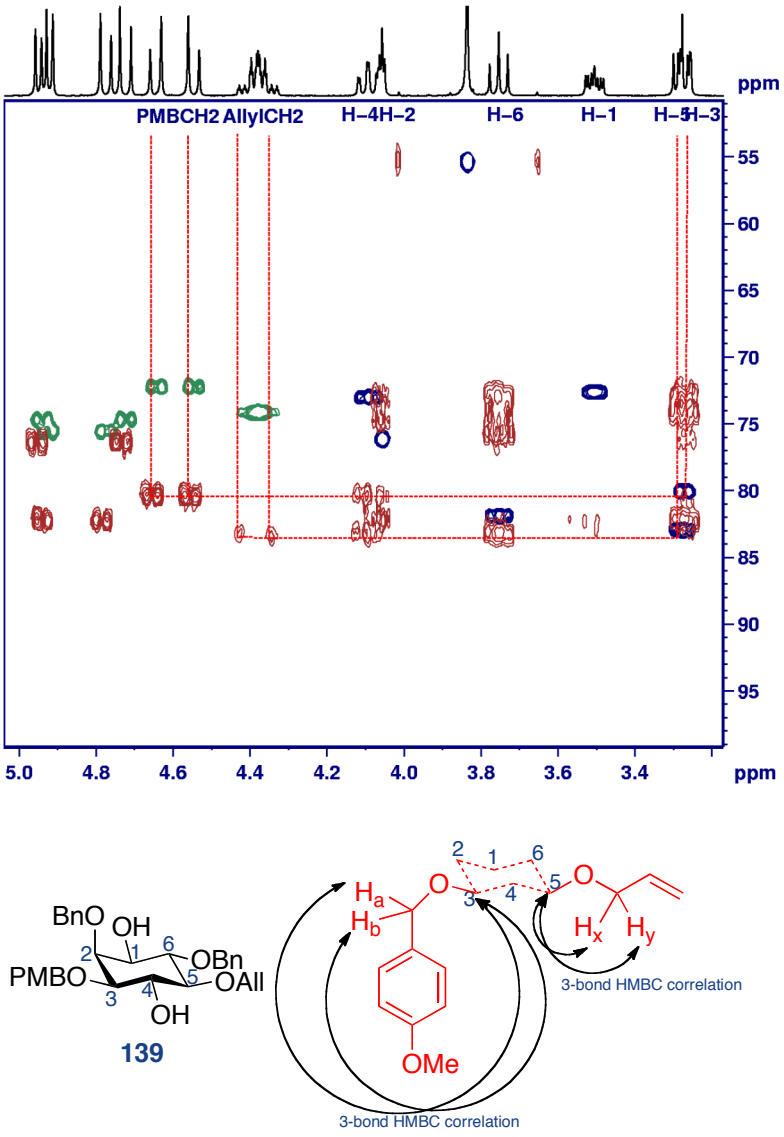


Figure 3.4. ^{1}H-^{13}C hetronuclear single quantum coherence (HSQC [multiplicity edited, CH_2 signals in green and CH and CH_3 signals in blue]) overlayed with ^{1}H-^{13}C hetronuclear multiple bond coherence (HMBC [signals in red]) of diol **139**.

Having successfully installed the desired PMB ether reliably on the 3-position hydroxyl group, removal of the allylic ether to furnish triol **106** was attempted (**table 3.3**). Initial conditions using Wilkinson's catalyst to isomerise, followed by acidic cleavage gave the desired triol **106** in 49% yield (entry 1, **table 3.3**). Loss of material was observed as the propyl ether which is formed by reduction of the double bond and which is not hydrolysable under standard acidic conditions. Therefore the BuLi modified method was attempted, however after 6 hours under

reflux in the presence of the modified catalyst no isomerisation to the enol ether was observed (entry 2, **table 3.3**).

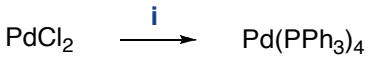
139 **106**

Entry	Conditions	Reaction Time	Yield
1	i. Wilkinson's catalyst, Hünig's base, EtOH, reflux ii. AcCl, MeOH, CH_2Cl_2, RT	8 h	49%
2	i. Wilkinson's catalyst, ^{n}BuLi, THF, -78 °C to reflux ii. AcCl, MeOH, CH_2Cl_2, RT	No reaction	n/a
3	$Pd(PPh_3)_4$, AcOH, RT	48 h	93%
4	$Pd(PPh_3)_4$, AcOH, 80 °C	6 h	89%

Table 3.3. Summary of conditions for the removal of allylic ether from intermediate **139**

A literature search was conducted in order to find alternative conditions that might effect the allyl removal with improved yields. Conditions published by Kusama and co workers in 1991,[100] described a simple procedure where treatment of a number of allylic ethers with catalytic $Pd(PPh_3)_4$ in glacial acetic acid furnished the desired alcohol in less than 6 h at 80 °C with excellent yield. Therefore, a solution of allylic ether **139** in glacial acetic acid was treated with $Pd(PPh_3)_4$ but due to the possible acid sensitivity of the PMB ether present in the molecule the reaction was conducted at room temperature as opposed to 80 °C. The reaction proceeded smoothly but was sluggish at room temperature requiring 48 h and almost stoichiometric $Pd(PPh_3)_4$ to complete. However, upon work up and chromatographic purification the desired triol **106** was achieved in 93% yield (entry 3, **table 3.3**). The reaction was repeated at 80 °C where completion was observed within 6 h and with an 89% isolated yield of triol **106**. Almost stoichiometric amounts of $Pd(PPh_3)_4$ were still required but no hydrolysis of the PMB ether was observed under the heated conditions (entry 4, **table 3.3**).

Small quantities of triphenylphosphine impurities were observed in the ^{1}H NMR of the product, although these were easily removed by re-crystallisation of triol **106** from ethyl acetate and petroleum ether. A further improvement in the purity of the reaction was found when using freshly made $Pd(PPh_3)_4$, which is made in one step from $PdCl_2$ by treatment with triphenylphosphine and hydrazine hydrate in DMSO (**scheme 3.19**).



Scheme 3.19. Synthesis of $Pd(PPh_3)_4$. *Reagents and conditions*: **i.** $PdCl_2$, PPh_3, DMSO, hydrazine hydrate, 140 °C → 125 °C, quantitative yield.

Although the regioselectivity of PMB ether **139** was fully assigned by NMR analysis, removal of the allylic ether gave triol **106** that was sufficiently crystalline enabling the acquisition of a single crystal X-ray structure (**fig. 3.5**). This confirmed the initial NMR assignment by clearly showing the relative position of the PMB ether at the 3-position.

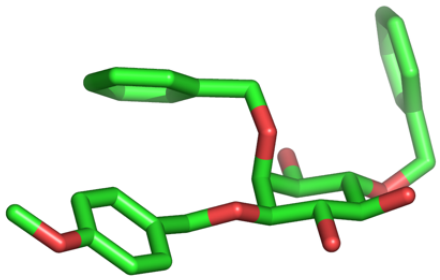
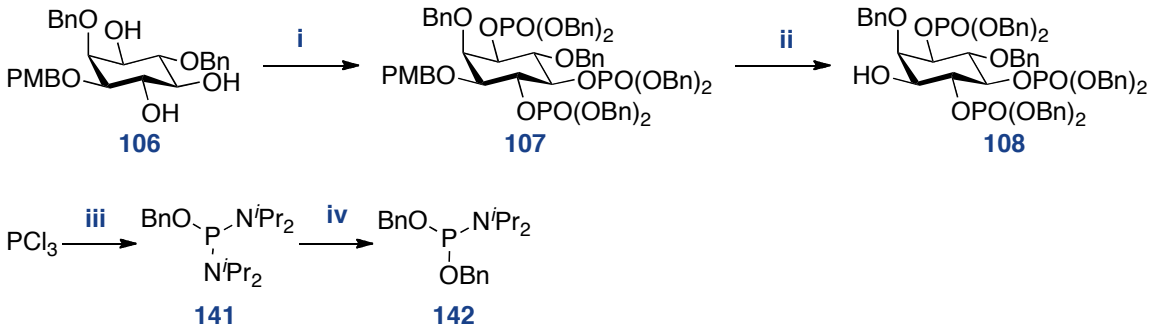


Figure 3.5. Single crystal X-ray structure of the triol **106** (C=green, O=red).

3.7. Synthesis of alcohol 108

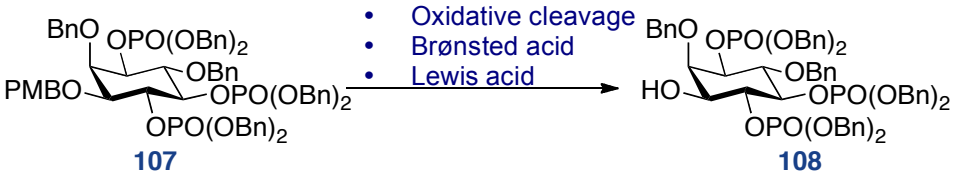
Having achieved a robust synthesis of the desired PMB ether **106**, installation of the fully benzyl protected phosphates to give the PMB protected trisphosphate **107** was required. A reliable method for the installation of fully benzyl protected phosphates, and one successfully utilised within the group for the synthesis of Ins(1,3,4,5)P_4 analogues, reacts the appropriate alcohol with bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine in the presence of catalytic 1*H*-tetrazole. This reaction gives the corresponding inositol phosphine, which is subsequently

oxidised with 3-chloroperoxybenzoic acid (*m*CPBA) to yield the desired benzylphosphate ester.



Scheme 3.20. Synthesis of alcohol **108**. *Reagents and conditions*: **i.** (a) bis(Benzyloxy)-*N*,*N*-diisopropylamino phosphine, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78°C 89% yield; **ii.** TMSCl, anisole, $SnCl_2$, CH_2Cl_2, RT, 19%-77% yield. Synthesis of bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142**; **iii.** PCl_3, pyridine, BnOH, diisopropylamine, Et_2O, 81% yield; **iv.** 1*H*-tetrazole, BnOH, CH_2Cl_2, 88% yield.

Thus, bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine was successfully prepared using a literature procedure from phosphorus trichloride (PCl_3) **scheme 3.20**. PCl_3 was firstly cautiously reacted with 1 equivalent of BnOH followed by excess diisopropyl amine in diethyl ether and stoichiometric pyridine to give the desired benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **141**. No purification of **141** was attempted so the crude material was carefully reacted with 1 equivalent of BnOH and 1*H*-tetrazole in CH_2Cl_2, purification by flash silica gel column chromatography gave the desired bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine reagent **142** in 88% yield (**scheme 3.20**). The triol **106** was reacted with excess bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** and catalytic 1*H*-tetrazole in CH_2Cl_2 at room temperature for 18 h. The crude reaction mixture was then oxidised with *m*CPBA at -78 °C and upon work up and chromatographic purification, the desired trisphosphate **107** was isolated in 89% yield (**scheme 3.20**). The subsequent step was to remove the PMB ether, furnishing the potentially versatile alcohol **108** (**scheme 3.21**). Widely used and reliable procedures for this transformation use oxidative cleavage using either ceric ammonium nitrate (CAN) or 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).



Scheme 3.21. Common methods for the removal of PMB ethers.

Work reported within the group successfully removed a PMB ether with CAN in the presence of multiple fully benzyl protected trisphosphates in high yield.[31,91,92] Therefore, **107** was treated with 2.5 eq of CAN at room temperature in MeCN/H_2O, this appeared to cleave the PMB ether as adjudged by TLC analysis. The reaction was sluggish and required further equivalents of CAN after 12 h. Isolation of the major product gave a sticky solid in 42% yield (**table 3.4**). ^{1}H NMR analysis indicated that the desired product had formed but both ^{1}H and ^{31}P NMR analysis showed impurities that could not be seen by TLC analysis. The reaction was repeated using half the amount of solvent, this appeared to greatly increase the rate and yield (73 %) of reaction and eliminated the need for extra CAN (**table 3.4**). Chromatographic purification followed by ^{1}H and ^{31}P NMR analysis showed an increase in the same impurities observed in the initial reaction. On close inspection of the NMR data these impurities were attributed the migration of the sterically strained 4-position protected phosphate migrating to the less constricted 3-position of the inositol ring (**fig. 3.6**).

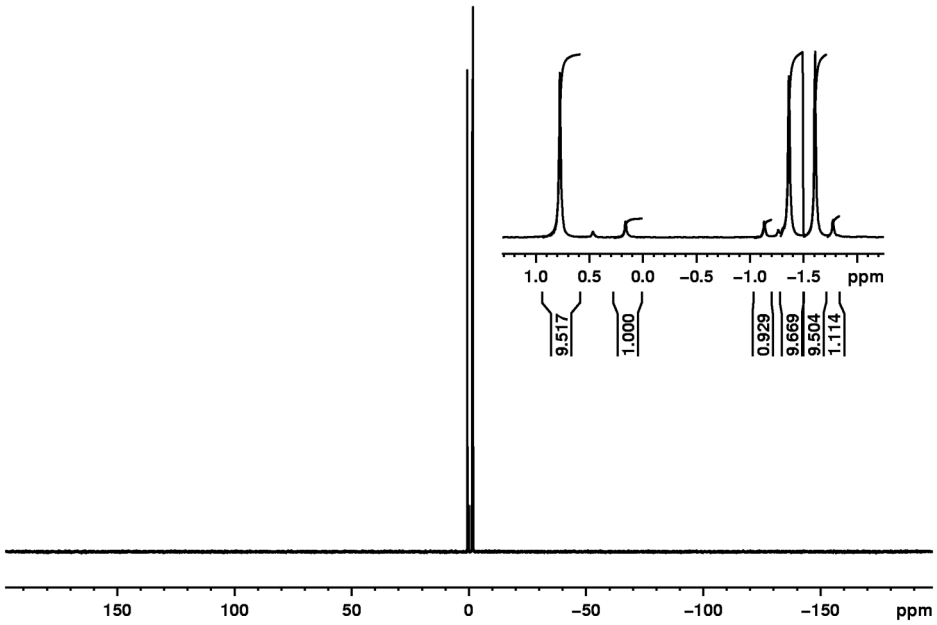


Figure 3.6. ^{31}P NMR spectrum of alcohol **108** after deprotection with CAN showing migration at a 9:1 ratio of non-migrated to migrated product.

Exhaustive silica gel column chromatography failed to separate the undesired regioisomer formed by the migration.

A pH measurement showed that the addition of CAN lowers the reaction pH to around pH 1. It was postulated that this strong acidity might be facilitating the migration. Different work conducted within the group also encountered problems due to the acidity caused by CAN, the solution in this case was to buffer the reaction at neutral pH, this did not appear to affect the reactivity of CAN and prevented product degradation. Therefore, a reaction was carried out where a pH 7 concentrated trisborate EDTA buffer was added to a solution of **107** prior to the addition of CAN, this proved to be effective in buffering the reaction to a pH 7, however, no reaction was observed. Dilution of the buffer solution simply failed to effectively buffer the reaction and as a result a pH of 1 was observed. This acidity, however, allowed the reaction to proceed but ^{1}H and ^{31}P NMR analysis of the products showed that migration had occurred.

As migration of the phosphates leads to inseparable regioisomers of **108**, different reaction conditions were needed in order to obtain **108** pure, the results of this study are summarised in **table 3.4**.

Type	Conditions	Conc. of 12 / mg ml^{-1}	Time	Yield	Migration
Oxidative	CAN, MeCN/H_2O, RT	10	24 h	42%	Yes
	CAN, MeCN/H_2O, RT	25	12 h	73%	Yes
	CAN, conc. Trisborate EDTA pH 7 buffer, MeCN/H_2O, RT	10	48 h	No reaction, staring material reclaimed	N/A
	CAN, 5× diluted Trisborate EDTA pH 7 buffer, MeCN/H_2O, RT	25	24	Complex mixture	Yes
	CAN, AcCN/H_2O, 0 °C	15	12 h	30%	Yes
	DDQ, CH_2Cl_2/H_2O, RT, acid work-up	10	12 h	70%	Yes (moderate)
	DDQ, CH_2Cl_2/H_2O, RT, basic work-up	10	12 h	-	Yes (considerable)
	DDQ, CH_2Cl_2/H_2O, RT, neutral work-up	10	12 h	-	Yes
	I_2, MeOH, reflux	30	12 h	Complex mixture	Complex mixture
Acidic	TMSI (2 eq), $CHCl_3$, RT	78	24 h	No reaction, staring material reclaimed	N/A
	AcOH, 90 °C	25	18 h	Complex mixture	Complex mixture
Lewis Acid	$ZrCl_4$, MeCN	10	24 h	No reaction, staring material reclaimed	N/A
	TMSCl, anisole, $SnCl_2$, CH_2Cl_2, RT	10	15 min	15%	Yes
	TMSCl, anisole, $SnCl_2$, CH_2Cl_2, RT	5	3 h	Variable 77%-19%	No

Table 2.4. Summary of conditions for the synthesis of **108**.

Cooling the reaction of CAN with PMB ether **107** had the effect of significantly slowing the reaction but unfortunately had negligible effect on the amount of phosphate migration observed. DDQ effected the PMB cleavage more rapidly than CAN but the initial conditions gave considerable migrated products. The work-up procedure for this reaction washes the organic component with a $NaHCO_3$ solution, this removes a large amount of the phenolic by-products as the phenolate salts. Basic conditions, albeit strongly basic (such as NaH or NaOH), are known to cause considerable phosphate migrations. By changing

the work-up procedure of the DDQ reaction to a weakly acidic one, purification was harder but migration was significantly reduced from an 18:1 ratio to a 4:1 ratio of migrated to non-migrated product (**fig. 3.7**). This result indicates that even weak base should be avoided. Neutral work-up conditions for the DDQ cleavage still showed migration indicating that this is inherent in using DDQ.

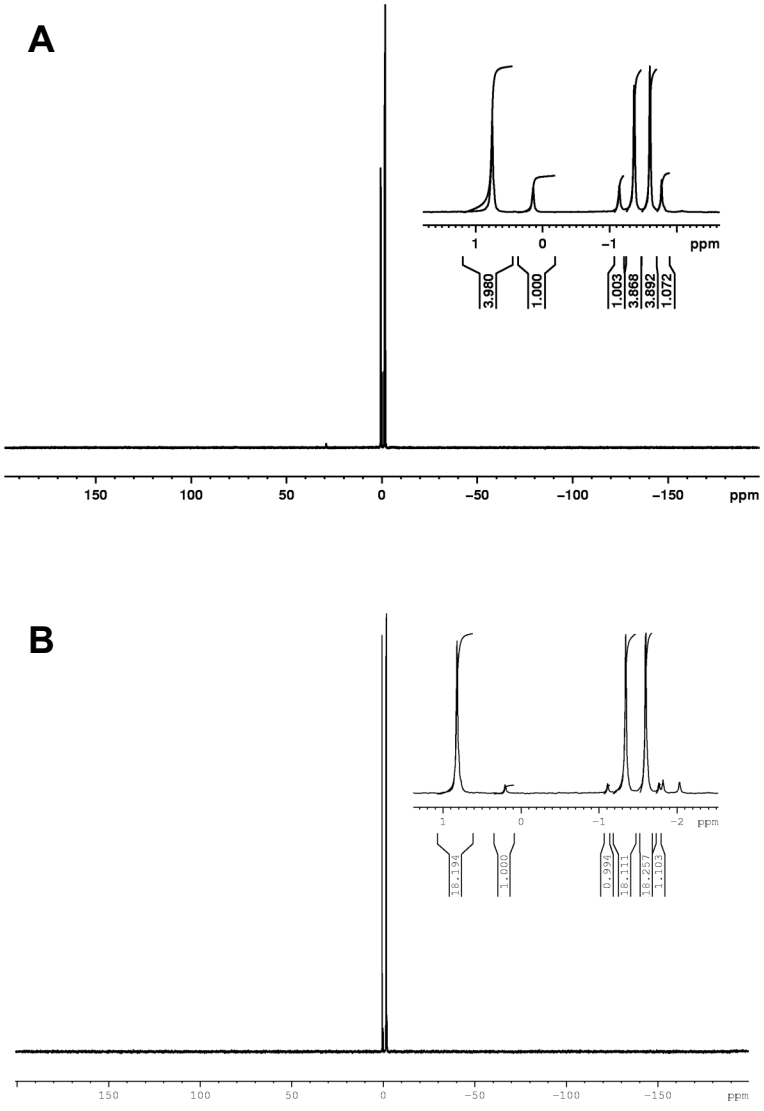
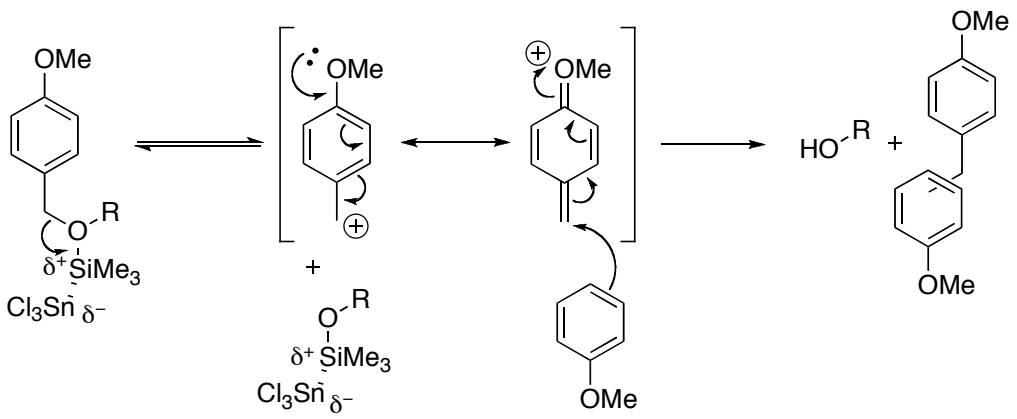


Figure 3.7. Showing the decreased migration from a basic work-up procedure **A** compared to an acid work-up **B**.

With the oxidative cleavage of the PMB ether leading to phosphate migrations, another avenue had to be explored. Many methods for the selective removal of

PMB ethers involve the activation of the PMB group *via* Lewis acid coordination followed by cleavage either hydrolytically or with a sacrificial nucleophile. Of the Lewis acid catalysed methods known, a procedure described by Ozaki and co-workers was attempted on this system.[101] This method involved the reaction of PMB ether with TMSCl, anisole and a catalytic amount of $SnCl_2$. The reaction was very fast reaching completion within 15 minutes. It did, however, only yield 15% crude material and also showed migration. Nevertheless, it was postulated that diluting the reaction might slow it enough to a controllable rate and also reduce the occurrence of phosphate group migration. The reaction was, therefore, repeated with a two-fold dilution. This dilution appeared to significantly reduce the reaction rate, which went to completion within 3 h, purification and isolation initially gave the desired product in 77% yield with no detectable migrated product. Unfortunately, subsequent reactions using the same conditions on larger scale diminished the yield significantly, only isolating 19-40% of the clean product free from migration. It was observed that the reaction time was also increased on the larger scale taking up to 6 h to complete. TLC analysis showed the presence of baseline impurities, isolation and analysis, by ^{1}H and ^{31}P NMR were of poor quality due to the amount of overlap so no firm structure could be postulated from the NMR alone. However, mass spectrometry provided strong evidence that multiple de-benzylations were occurring during the reaction, showing a negative ion mass corresponding to M-Bn.

The proposed mechanism for the PMB ether cleavage is the formation of a $SnCl_2$-TMSCl complex that coordinates with the ether oxygen activating the benzylic position towards the formation of the cationic intermediate, which is stabilised in the case of PMB ether, this is then trapped out by the sacrificial nucleophile anisole (**scheme 3.22**).[101]

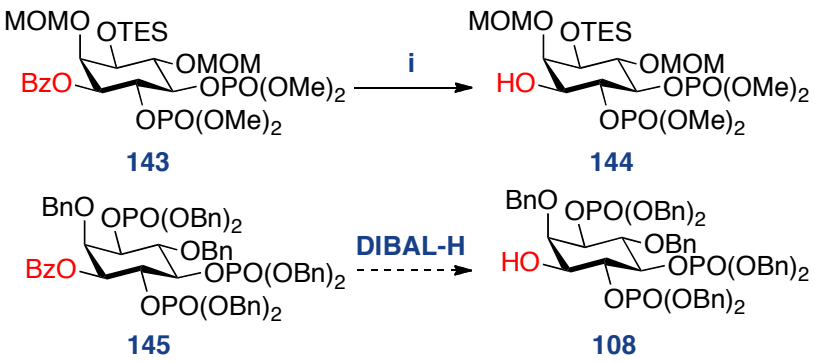


Scheme 3.22. Mechanism of PMB cleavage using TMSCl, $SnCl_2$ and anisole.[101]

The original paper reported benzyl ethers being stable under their conditions. This stability is presumably because the forming benzyl benzylic cation is not stabilised compared with PMB benzylic cation resulting in PMB groups reacting much faster. However, in our system, because of the steric strain experienced by the vicinal phosphates, the loss of a benzyl group must be energetically favourable thus shifting the equilibrium of the process over to the more favourable debenzylated intermediate. With extended reaction times on larger scale, the loss of material due to de-benzylation reduces the yield of the reaction. In addition, it was not possible to reliably repeat the reaction on the same scale that produced the initial high yield, therefore making this unsuitable for producing enough of the desired alcohol **108** for further elaboration to final compound precursors.

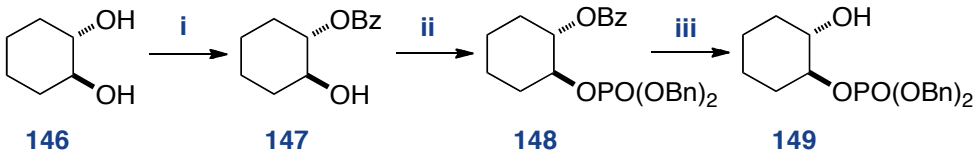
3.8. Synthesis of benzoyl protected trisphosphate 145

Given the poor yield and unreliable nature of the PMB deprotections, it was decided that an alternative protecting group strategy was required. A literature search found a procedure whereby a benzoyl ester was removed using DIBALH from the 3-position hydroxy, in the presence of vicinal protected phosphates, in high yield and with no apparent phosphate migration (**scheme 3.23**).[102]



Scheme 3.23. Deprotection of benzoyl ester in the presence of vicinal phosphate diesters.[102] *Reagents and conditions*: **i.** DIBALH, CH_2Cl_2, -78 °C, 84% yield.

The vicinal phosphate esters in the published example are methyl protected, so it was a concern that DIBALH might react with the benzyl protected phosphates of the desired compound. Hence, to check the stability of the bisbenzyl phosphate esters in the presence of DIBALH, a model system was used (**scheme 3.24**). An additional concern is that DIBALH might facilitate migrations of the benzyl protected phosphates, however, the benefit of the method is that reactions are conducted at -78 °C so it was hoped that this temperature might prevent migration.

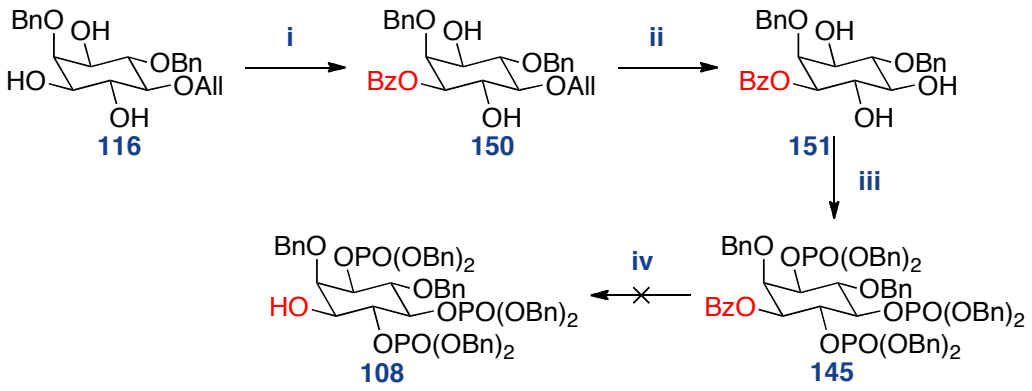


Scheme 3.24. Model system for the deprotection of benzoyl ester in the presence of fully benzyl protected phosphate ester. *Reagents and conditions*: **i.** Bu_2SnO, toluene, benzoyl chloride, reflux, 50% yield; **ii.** (a) bis(Benzyloxy)-*N*,*N*-diisopropylamino phosphine, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78°C, 56% yield; **iii.** DIBALH, CH_2Cl_2, -78°C, 87% yield.

Thus *trans*-cyclohexane diol **146** was mono protected as the benzoylester by reaction with dibutyltin oxide and benzoyl chloride under reflux in toluene, with a Dean-Stark apparatus attached for the azeotropic removal of water. This reaction gave the desired monobenzoyl ester **147** in 50% yield. **147** was then reacted with excess bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** and catalytic 1*H*-tetrazole in CH_2Cl_2 at room temperature for 18 h. The crude reaction mixture was then oxidised with *m*CPBA at -78 °C and, upon work up and

chromatographic purification, the desired monophosphate **148** was isolated in 56% yield. A solution of monophosphate **148** in CH_2Cl_2 was cooled to -78 °C and treated with DIBALH, consumption of the starting material was observed within 15 min, corresponding with the formation of a new product observed by TLC analysis. The reaction was quenched at -78 °C and after work-up and purification, analysis showed that alcohol **149** was isolated in 87% yield. No debenzylation of the protected phosphate ester was observed (**scheme 3.24**).

As a result of the test reaction, it was desirable to attempt a reaction on the real substrate. In order not to waste enantiomerically pure material the decision was made to conduct the synthesis of the desired benzoyl protected trisphosphate **145** racemically (**scheme 3.25**).



Scheme 3.25. Synthesis of alcohol **108** using benzoyl protection of 3-position hydroxyl. *Reagents and conditions*: **i.** Bu_2SnO, toluene, benzoyl chloride, reflux, 60% yield; **ii.** $Pd(PPh_3)_4$, AcOH, RT, 86% yield; **iii.** (a) bis(Benzyloxy)-*N*,*N*-diisopropylamino phosphine, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperbenzoic acid, -78°C, 56% yield; **iv.** DIBALH, CH_2Cl_2, -78°C.

Therefore racemic triol **116** was mono protected in a similar fashion as before, by reaction of **116** with dibutyltin oxide and benzoyl chloride in refluxing toluene with a Soxhlet apparatus containing 3 Å molecular sieves for the removal of water. This gave the desired monobenzoyl ester **150** in 60% yield. The regiochemistry was assigned by ^{1}H NMR analysis as the 3-position benzoyl ester, no other regioisomers were observed from the reaction. Removal of the allylic ether to furnish the triol **151** was achieved in 86% yield by stirring diol **150** with $Pd(PPh_3)_4$ in glacial acetic acid at room temperature. Reaction of **151** with excess bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** and 1*H*-tetrazole in CH_2Cl_2

at room temperature for 18 h gave the crude phosphine that was then oxidised with *m*CPBA at -78 °C. Upon work up and chromatographic purification the desired trisphosphate **145** was isolated in 70% yield. A solution of trisphosphate **145** in CH_2Cl_2 was therefore cooled to -78 °C and treated with DIBALH, an attempt to monitor phosphate migration during the reaction by ^{31}P NMR analysis failed to give clear phosphorus signals probably due to metal ion coordination by the aluminate species present in the reaction mixture. Nevertheless consumption of the starting material was observed after 2.5 h. Work-up and isolation of the product showed that removal of the benzoyl ester group had been achieved but a significant amount of phosphate group migration had also occurred.

3.9. Summary

The synthesis of 3-position modified Ins(1,3,4,5)P_4 derivatives requires masking the 3-position hydroxyl group with an appropriate protecting group. The protection of the 3-position hydroxyl group using stannylene acetal chemistry, with a PMB ether, was initially found to be difficult when protecting groups were present at the 1-position. The poor reaction yields and difficult regioisomer separations, experienced with early reactions, were overcome when it was found that the stannylene acetal chemistry works well on triol **138**. It was found that the PMB protecting group, could be installed onto the 3-position hydroxyl of triol **138**, in remarkably good yield and good selectivity. The success of this reaction allowed for the reliable synthesis of PMB trisphosphate **107**. However, reaction conditions for the subsequent removal of the PMB group, to furnish the desired alcohol **108**, were not reliable, often causing phosphate group migration or debenzylation. This prompted the exploration for a different protecting group; the literature suggested that the benzoyl ester might be a good alternate.[102] The installation of the benzoyl ester protecting group, onto the 3-position hydroxyl group of triol **116**, worked in higher yield and better selectivity, compared with the PMB ether. This successful reaction allowed for the reliable synthesis of benzoyl ester trisphosphate **145**. However, the published conditions for the DIBALH

mediated removal of the ester, to furnish alcohol **108**, resulted in a significant amount of phosphate migration.

It has become clear that in order to make multiple derivatives of Ins(1,3,4,5)P_4 modified at the 3-position, it is crucial to have a robust synthesis of alcohol **108**. This requires protection of the 3-position hydroxyl group with a protecting group that is easily removed under mild conditions, avoiding strong acid or base. There is seemingly a strong thermodynamically favourable arrangement of the benzylphosphate esters that surround the inositol ring. Therefore, in order to minimise the steric strain experience by the vicinal phosphates of alcohol **108** it seems only to require moderately activating conditions to promote phosphate migration or de-benzylation.

4. Results and Discussion Part 2: The synthesis of 5-position modified Ins(1,3,4,5)P_3 analogues

4.1. Synthetic Targets

With work on-going towards the synthesis of 3-position modified PtdIns(3,4,5)P_3 analogues, the focus of the project shifted towards the synthesis of 5-position modified PtdIns(3,4,5)P_3 derivatives. Again, completing mono-substitution of all the phosphate groups surrounding the inositol ring of Ins(1,3,4,5)P_4 is desirable as it will allow us to assess the importance of each phosphate for binding to specific PH domains. Knowing the positions where modification is well tolerated will allow us to synthesise more targeted analogues, hopefully giving extra binding affinity and improved selectivity towards specific PH domains.

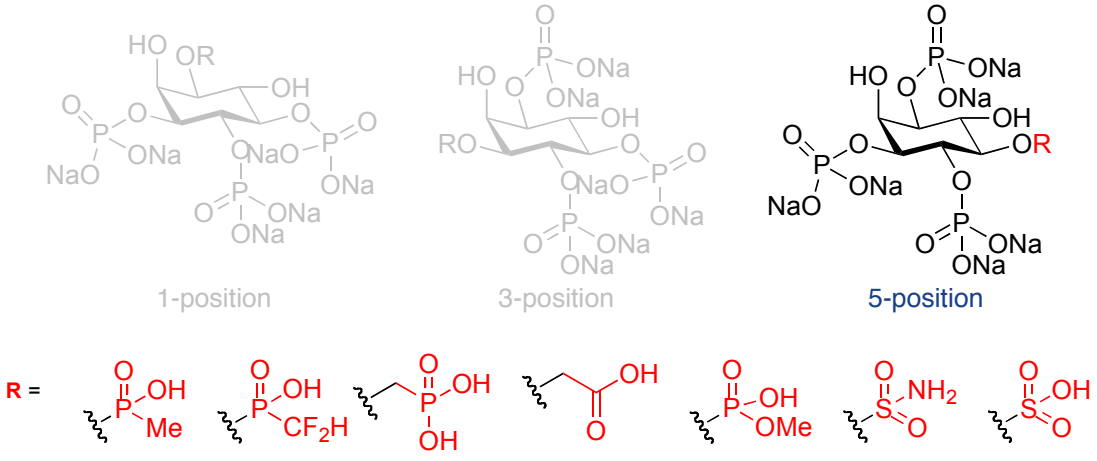
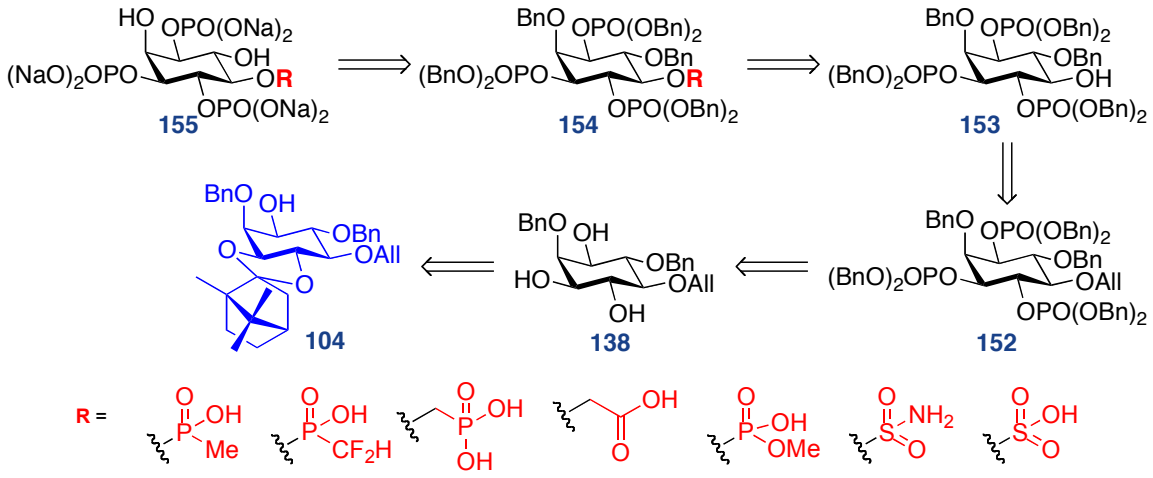


Figure 4.1. 5-Position modified targets.

As before, due to the basic nature of the PKB-PH domain it is desirable to install acidic isosteric phosphate mimics such as those outlined in **fig. 4.1**. These isosteres should bear a reduced charge compared to that of the native phosphate. It is also desirable to install 5-position phosphate replacements analogous to those already synthesised at the 1- and 4-positions, such as the methylene phosphonate **24**, sulfate **25**, and sulfate **28**; this strategy will allow for the direct comparison between regioisomers.

4.2. Retrosynthesis

A general retrosynthetic analysis for 5-position modified Ins(1,3,4,5)P_4 derivatives, is represented in **scheme 4.1**. The approach that was used for the work towards 3-position derivatives was employed for the synthesis of 5-position derivatives. Thus, the desired final compounds **155** would be achieved from a global de-protection of fully benzyl protected precursor compound **154**.



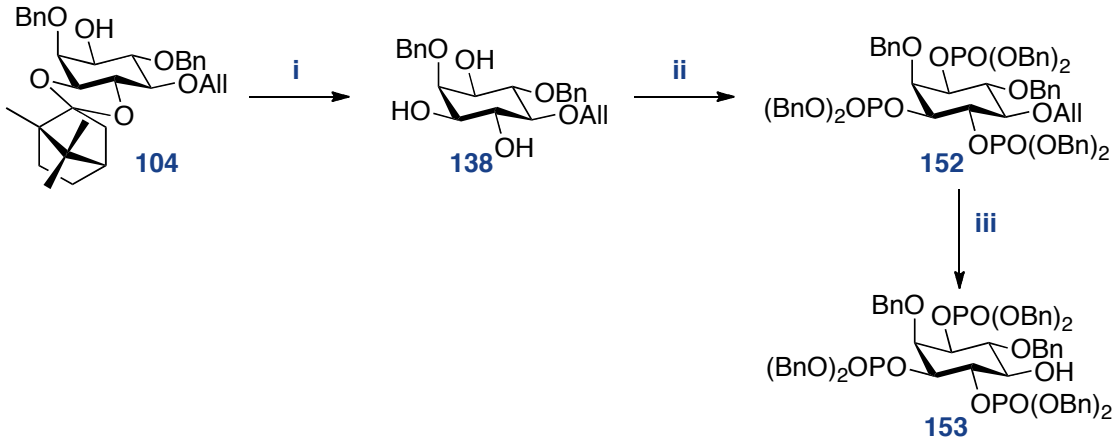
Scheme 4.1. General retrosynthesis for 5-position InsP_4 derivatives.

It was desirable to synthesise the 5-position alcohol trisphosphate **153**, which can potentially undergo multiple derivatisations to give a range of precursors **154**. Masking the 5-position alcohol requires the presence of an appropriately orthogonal protecting group, the simplest way to achieve this is to preserve the allyl protecting group that is already present in enantiopure alcohol **104**. Phosphitylation of enantiopure triol **138** would then give the orthogonally protected trisphosphate **152**. It was hoped that conditions for removal of the allyl protecting group from **152** would furnish alcohol **153** reliably, in high yield and, importantly, free from phosphate migration.

4.3. Synthesis of the trisphosphate 153

The synthesis of enantiomerically pure alcohol **104** is described in chapter 2 and was conducted on multi-gram scale to provide material for the development of the 5-position synthesis.

The synthesis of the allyl-protected trisphosphate **152** proved to be straightforward (**scheme 4.2**). Firstly, removal of the camphor auxiliary from alcohol **104** was achieved by acidic methanolysis, to give the enantiomerically pure triol **138**, in 76% yield. Triol **138** was reacted with excess bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** and catalytic 1*H*-tetrazole in CH_2Cl_2 at room temperature for 18 h. The crude reaction mixture was oxidised with *m*CPBA at -78 °C and, upon work-up and chromatographic purification, the desired trisphosphate **152** was isolated in 89% yield (**scheme 4.2**).



Scheme 4.2. Synthesis of alcohol **153**. *Reagents and conditions*: **i.** AcCl, CH_2Cl_2/MeOH, RT, 76% yield; **ii.** (a) bis(Benzyloxy)-*N*,*N*-diisopropylamino phosphine, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78°C, 89% yield; **iii.** $PdCl_2$, MeOH, RT, 65% yield.

Having isolated trisphosphate **152**, it was important to have optimal conditions for the removal of the allyl protecting group. In the first instance, using the conditions optimised for allyl removal of **139** described in chapter 2, a solution of trisphosphate **152** in glacial acetic acid was treated with freshly prepared $Pd(PPh_3)_4$ at room temperature. Complete consumption of the starting material was observed after 18 h, requiring over 2 equivalents of the catalyst. These conditions led to the appearance of new baseline products, as observed by TLC

analysis. Work-up and isolation of the major product, however, gave the desired 5-position alcohol in 55% yield assigned by ^{1}H NMR analysis. Gratifyingly, the ^{1}H and ^{31}P NMR assignment showed that the product was free from phosphate migration (**fig. 4.2**).

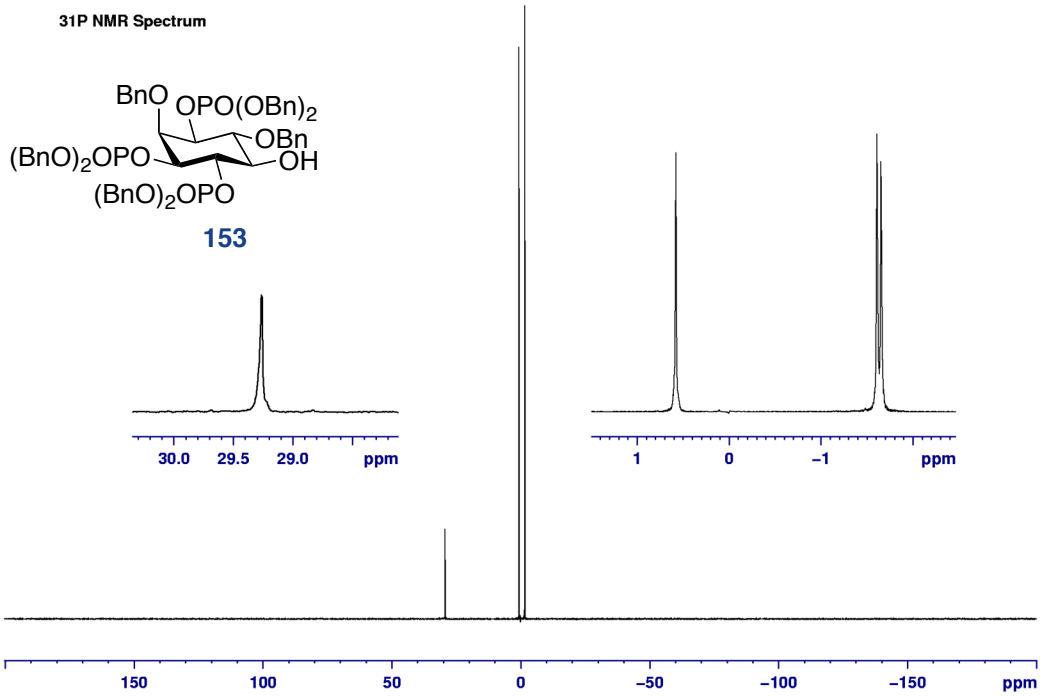


Figure 4.2. ^{31}P NMR spectrum of 5-position alcohol **153** showing the three main signals corresponding to the phosphate groups and an additional triphenyl phosphine impurity.

Due to the high polarity of the trisphosphate, purification *via* silica gel column chromatography failed to effectively remove all of the triphenyl phosphine from the catalyst. A signal for this impurity can be seen in the ^{31}P NMR spectrum at 29.3 ppm (**fig. 4.2**). Multiple chromatographic columns eventually yielded the pure substrate, however, this further diminished the yield of the product. For a robust synthesis a higher yielding and more reliable allyl deprotection was desirable, therefore, some optimisation studies were conducted, the results of which are summarised in **table 4.1**. As was observed with the synthesis of triol **139** in chapter 3, it was hoped that heating might boost the rate of reaction and thus reduce the number of equivalents of $Pd(PPh_3)_4$ required, thus reducing the

amount of PPh_3 impurity. Indeed, by heating the reaction to 80 ºC complete consumption of starting material was observed within 6 h with only 0.5 equivalents of $Pd(PPh_3)_4$ required. However, equally lengthy column chromatography was required to remove the triphenyl phosphine impurities, ultimately with a reduction in the isolated yield (entry 2, **table 4.1**). Stripping the chromatographic column with a high polarity flush obtained material present at the base-line of the TLC analysis. Mass spectrometry of these products showed the presence of $[M+NH_4{\cdot}MeCN]^+$ in positive ionisation mode (**fig 4.3**) and $[M-H]^-$ in the negative ionisation mode for the mono de-benzylated alcohol, indicating that at least some loss of material was due to de-benzylation of the product.

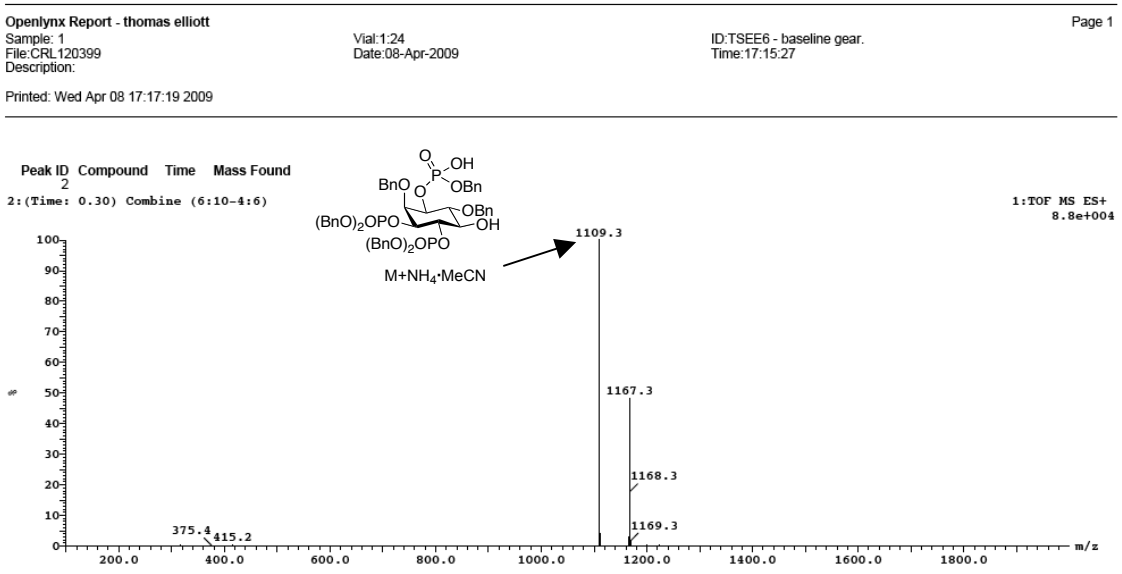


Figure 4.3. Mass spectrum of baseline material. Electrospray ionisation in positive mode, showing *m/z* 1109 $[M+NH_4{\cdot}MeCN]^+$ corresponding to debenzylated material.

Entry	Conditions	Time	Yield
1	$Pd(PPh_3)_4$ (2 eq), AcOH, RT	18 h	55%
2	$Pd(PPh_3)_4$ (0.5 eq), AcOH, 80 ºC	6 h	34%
3	$PdCl_2$ (8 eq), MeOH, RT	5 h	64%
4	$PdCl_2$ (2 eq), MeOH, RT (with oxidative work-up)	5 h	65%

Table 4.1. Summary of condition for the removal of allylic ether from compound **152**.

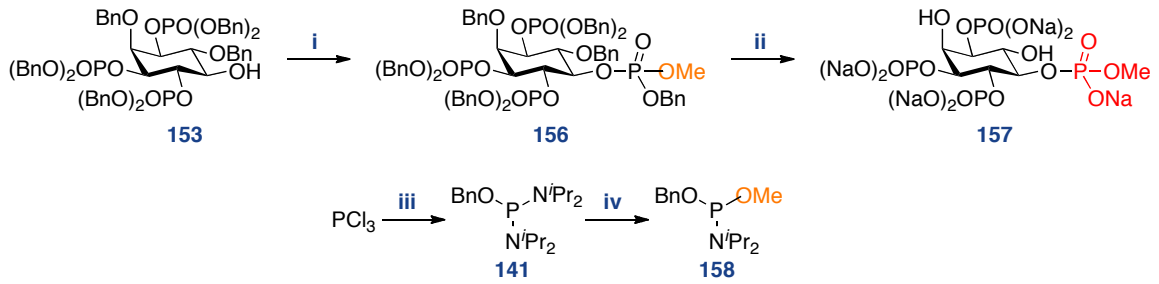
A search of the literature to find alternative methods for a mild cleavage of allylic ethers was conducted. This search found many conditions that use palladium chloride ($PdCl_2$) for the removal of allylic ethers from various oligo-saccharides that contain diverse functionality.[103-105] Initially, using the conditions reported by Mandai *et al.*,[103] a solution of allyl ether **152** in methanol was treated with 8 equivalents of $PdCl_2$; the reaction proceeded smoothly to completion within 5 h. Purification, however, proved troublesome requiring three silica gel chromatographic columns to yield pure material. It was subsequently found that only 2 equivalents of palladium were necessary to perform the deprotection with no increase in reaction time. An oxidative work-up procedure for the removal of ruthenium residues is described (*vide infra*). It was postulated that the same work-up procedure might help with the removal of palladium residues from this reaction. Therefore, the organic fraction of the aqueous work-up was washed with an aqueous solution of hydrogen peroxide, this resulted in considerable effervescence and some dark precipitate, which was removed by filtration. However, this wash significantly reduced the colouration of the organic fraction and ultimately only a single flash chromatographic column was required to obtain the desired alcohol **153** in an equivalent yield (entry 4, **table 4.1**). In the ruthenium example (*vide infra*), the hydrogen peroxide oxidises the metal to give the insoluble ruthenium(IV) oxide which is removed by filtration. In this case it could be possible that palladium(II) chloride is oxidised to a water soluble palladium(IV) species or potentially the palladium(II) chloride could undergo hydrolysis to form hydrated palladium(II) oxide. This reaction was repeatable; however, on a larger-scale the reaction time was extended and significant de-

benzylation was observed. Therefore, to produce larger quantities of the desired alcohol **153**, several small-scale reactions were conducted in parallel; once complete they were combined for work-up and purification, which avoided significant loss of material through de-benzylation due to extended reaction time.

4.4. Synthesis of the methyl phosphate ester 157

Having developed a reliable synthesis of alcohol **153**, studies could begin towards the incorporation of isosteric phosphate replacements. As described in chapter 2, it was desirable to incorporate groups with a reduced charge. It was also very important that conditions used to install the isosteres were mild. The presence of the three protected phosphates in alcohol **153** provided challenges to overcome when installing the desired phosphate isosteres. Firstly, steric crowding around the inositol ring would likely limit the size of the functionality that could be installed at the free hydroxyl. Secondly, relief from the steric strain experienced by the trisphosphate might occur *via* phosphate migration or debenzylation, seen during the synthesis of both the 3-position and 5-position alcohols, **108** and **153**. Therefore, it was desirable to find mild reaction conditions that would avoid these potential tranformations. From the work carried out for the synthesis of the 3- and 5-position alcohols it was known that strong base, strong Brønsted and Lewis acids should be avoided.

It was, therefore, envisioned that a simple and mild method to synthesise an interesting isostere would be to incorporate the benzyl protected methyl phosphate ester. This synthesis could be achieved by reaction with the appropriate phosphoramidite **159**, followed by oxidation which, upon global deprotection, would yield the desired methyl phosphate ester **157** (**scheme 4.3**). Phosphoramidite **158** was, therefore, prepared from PCl_3 using a modification of conditions published by Bannwarth in 1987.[106] The benzyloxy bis(*N*,*N*-diisopropylamino)phosphoramidite **141** was prepared as described in chapter 3, then treated with 1 equivalent methanol in the presence of 1*H*-tetrazole to give the desired phosphoramidite **158** in 76% yield (**scheme 4.3**).



Scheme 4.3. Synthesis of the 5-position methyl phosphate ester, Ins(1,3,4,5)P_4 derivative. *Reagents and conditions*: **i.** (a) Phosphoramidite **158**, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78°C, 79% yield; **ii.** $NaHCO_3$, Pd black, H_2, $^tBuOH/H_2O$, RT, 83% yield. Synthesis of phosphoramidite **158**. *Reagents and conditions*: **iii.** PCl_3, Py, BnOH, diisopropylamine, Et_2O, 81% yield; **iv.** 1*H*-tetrazole, MeOH, CH_2Cl_2, 76% yield.

Phosphoramidite **158**, in excess, was then reacted with alcohol **153** in the presence of 1*H*-tetrazole at room temperature. Once consumption of the starting material was observed, the reaction was cooled to -78 °C and the crude phosphite oxidised, using *m*CPBA, to give the phosphate. Work-up and purification gave the desired methyl phosphate ester **156** in 79% yield as a 1.3:1 mixture of diastereomers and importantly without any phosphate migration, as adjudged by ^1H and ^{31}P NMR spectroscopy (**fig. 4.4**). Figure **4.4** shows the four phosphorus signals of the 31P NMR spectrum, which correspond to each phosphate of **156**, it can be seen that each signal is split into what look like doublets. This splitting corresponds to the major and minor diastereomers of **156**, formed by the reaction of alcohol **153** with the racemic phosphoramidite **158**. From the observed diastereomeric ratio, it can be seen that the reaction does not form a completely racemic mixture, indicating that the alcohol **153** imparts some stereo-control on the formation of the phosphite.

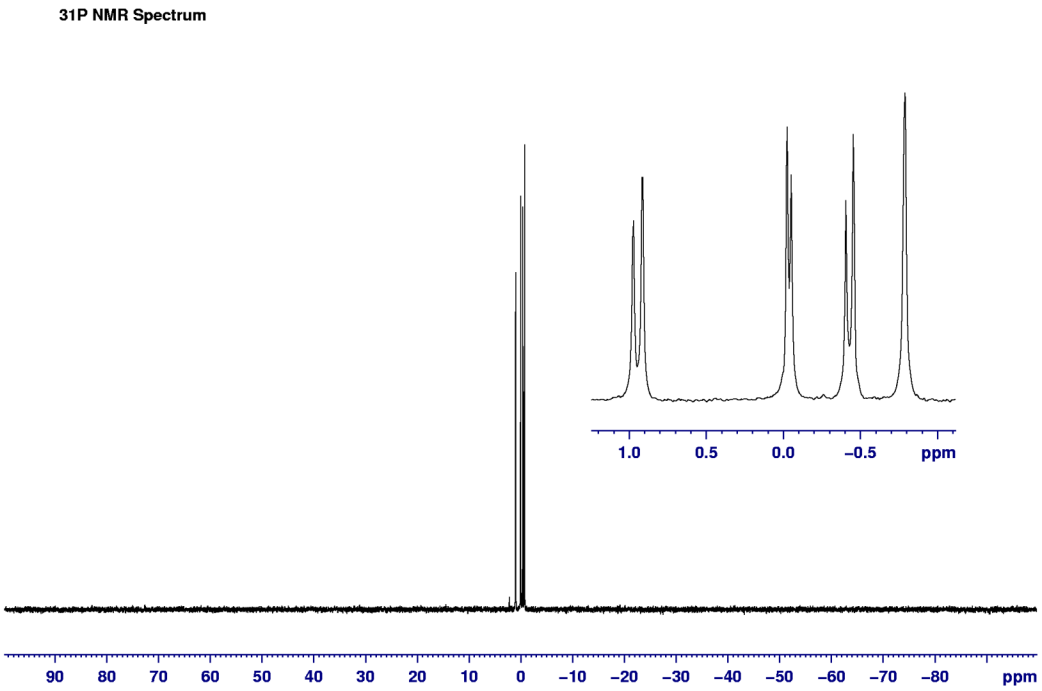


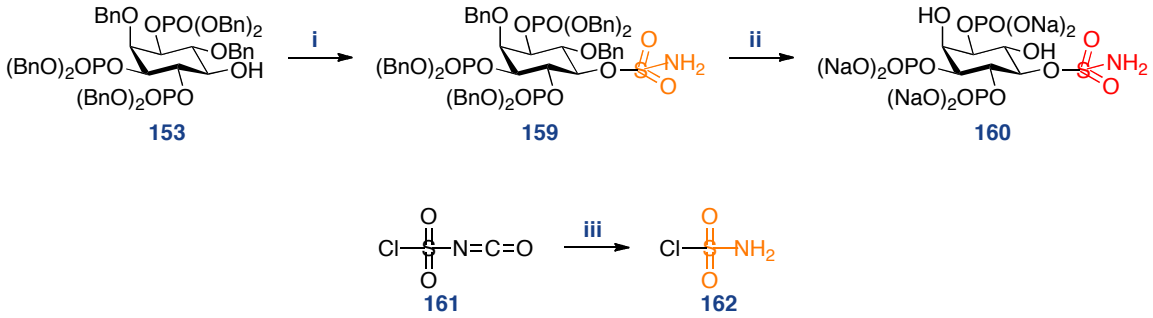
Figure 4.5. ^{31}P NMR of methylphosphate ester **156**, showing the four phosphorus signals corresponding to each phospate of **156**. Each signal is split into two, corresponding to the major and minor compounds of the diastereomeric mixture.

The global deprotection of the benzyl protecting groups proceeded smoothly. Pd-black catalysed hydrogenolysis was conducted in the presence of $NaHCO_3$ to give the presumed heptasodium salt. Isolation of the product gave the final compound **157** in 83% yield without the need for further purification.

4.5. Synthesis of Sulfamate 160

A common isosteric replacement for phosphates is a sulfamate group. This group can potentially act both as a hydrogen bond acceptor through the lone pairs of electrons on the oxygen and nitrogen atoms, or as a hydrogen bond donor through the hydrogens bonded to nitrogen. Common methods for sulfamoylation use sulfamoyl chloride with excess base and, when conducted in DMF, can produce undesired side products. However, an effective procedure for the sulfamoylation of a wide range of alcohols was reported by Okada *et al.* in

2000.[107] This method simply involves treating the alcohol or phenol with sulfamoyl chloride in dimethyl acetamide (DMA); giving the desired sulfamate with excellent conversion and high isolated yields. The DMA in this case acts as both reaction solvent and base.



Scheme 4.4. Synthesis of the 5-position sulfamate Ins(1,3,4,5)P_4 derivative. *Reagents and conditions*: **i.** Sulfamoyl chloride **162**, DMA, 0 °C → RT, 50% yield; **ii.** $NaHCO_3$, Pd black, H_2, $^tBuOH/H_2O$, RT, 84%. Synthesis of sulfamoyl chloride **162**. *Reagents and conditions*: **iii.** Formic acid, CH_2Cl_2, 0 °C, used as a 2.50 M solution in CH_2Cl_2.

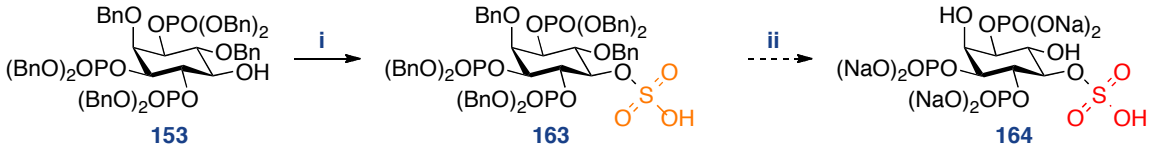
Sulfamoyl chloride is highly unstable, so sulfamoyl chloride was freshly prepared from chlorosulfonyl isocyanate (CSI), by treatment with formic acid at 0 °C. This reaction gave the crude sulfamoyl chloride, which was used as a 2.50 M solution in CH_2Cl_2 without further purification (**scheme 4.4**).

A solution of the trisphosphate **153** in DMA was cooled to 0 °C and treated with the freshly prepared sulfamoyl chloride **162**. The reaction proceeded smoothly to completion within 18 h, as adjudged by TLC analysis. Isolation and purification of the major product gave the desired sulfamate **159** in 50% yield (**scheme 4.4**). The global deprotection by hydrogenolysis of precursor **159**, using the conditions described previously but in the presence of 6 equivalents of $NaHCO_3$, gave the desired final compound **160** in 84% yield, without the need for further purification.

4.6. Attempted synthesis of sulfate 164

Having successfully synthesised the 5-position sulfamate derivative **160** it was desirable to make the 5-position sulfate **164**, for direct comparison. With the sulfate bearing a more acidic O-H than the N-H of the sulfamate, interesting information regarding the importance of pK_a towards PtdIns(3,4,5)P_3 binding

could be gained from these derivatives. In addition, it would be interesting to compare these compounds with the 4-position sulfate previously synthesised within the group.[31]



Scheme 4.4. Attempted synthesis of sulfate 5-position Ins(1,3,4,5)P_4 derivative. *Reagents and conditions*: **i.** SO_3·Pyridine complex, 50 °C, DMF; **ii.** $NaHCO_3$, Pd black, H_2, ^{t}BuOH/H_2O, RT.

Sulfation of alcohol **153** was attempted using conditions that were previously optimised within the group for the synthesis of the 4-position sulfate. Thus, alcohol **153** was stirred with excess sulfur trioxide pyridine complex (SO_3·Py) as a solution in DMF at 50 °C (**scheme 4.4**). Consumption of the starting material was observed by TLC analysis coinciding with the formation of a new major product. Mass spectrometry analysis found $[M+H]^+$ and $[M+NH_4{\cdot}MeCN]^+$ corresponding to the desired sulfate **163**. Unfortunately, isolation and purification proved difficult; silica gel column chromatography allowed isolation of the desired spot in 50% crude yield. ^{1}H and ^{31}P NMR analysis indicated that the desired sulfate had been formed; however, the product was not pure enough for the global deprotection step. Further attempts to purify the sulfate, through additional chromatography and ion exchange resin, diminished the yield of the material and failed to improve the quality of the compound. Compound **163** proved to be intrinsically unstable even when refrigerated indicated by the observation of peaks corresponding to $[M\text{-}SO_3H]^-$ and $[M\text{-}Bn]^-$ in the mass spectrum of the material as well as the observation of more polar impurities and starting material by TLC analysis. This indicated the compound was breaking down both through the loss of the sulfate moiety and the debenzylation of the phosphate diesters.

4.7. Attempted Synthesis of Methyl Phosphonate 166

Another interesting phosphate isostere is the methyl phosphonate group. The methyl phosphonate was successfully utilised by van Boom and co-workers during the published synthesis of methyl phosphonate containing Ins(1,4,5)P_3 analogues.[108,109]

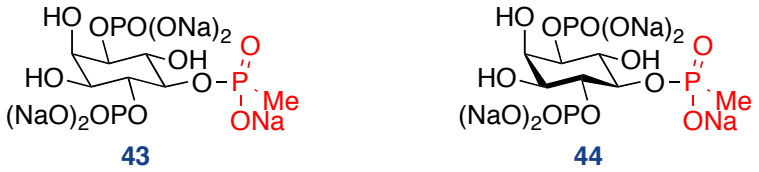
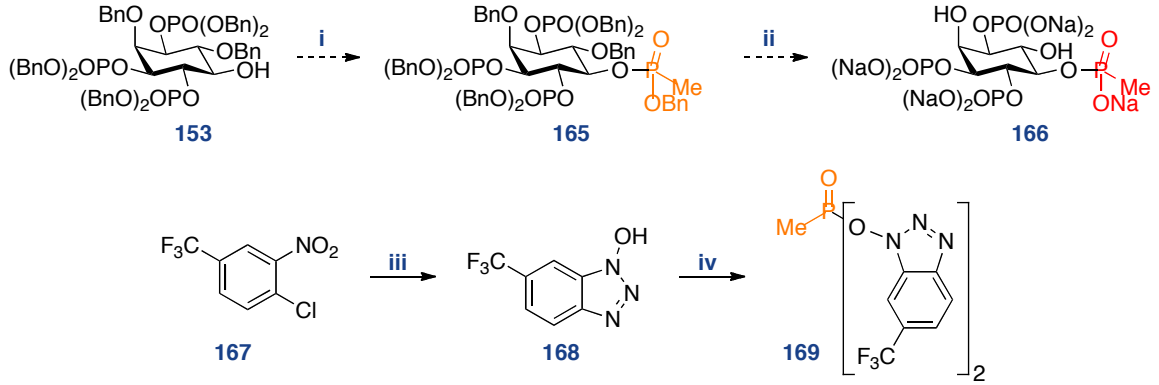


Figure 4.6. Methyl phosphonate containing Ins(1,4,5)P_3 analogues

Their work indicated that racemic analogue **43** (**fig. 4.6**) showed weak antagonistic activity against Ins(1,4,5)P_3 receptors, however, no biological data were published to support their claim. Subsequent unpublished work within our group successfully synthesised **43** in an enantiomerically pure fashion (**44**, **fig. 4.6**). Preliminary biological results suggest analogue **44**, behaves as an antagonist for Ins(1,4,5)P_3 receptors. For this reason it would be interesting to assess whether a similar modification would be tolerated within PH-domains.



Scheme 4.5. Attempted synthesis of the 5-position methylphosphonate Ins(1,3,4,5)P_4 derivative **166**. *Reagents and conditions*: **i.** (a) **169**, Py, 20 °C, 10 min, (b) BnOH; **ii.** $NaHCO_3$, Pd black, H_2, ^{t}BuOH/H_2O, RT. Synthesis of phosphonating reagent **169**. *Reagents and conditions*: **iii.** Hydrazine hydrate, ethanol, RT, 48% yield; **iv.** Methyl phosphonic dichloride, HOBT **168**, Py, dioxane, used crude as a 0.125M solution in dioxane.

To install the methyl phosphonate on alcohol **153**, the highly reactive phosphonating reagent **169** was made, following van Boom's original method (**scheme 4.5**).[108] Firstly, trifluoromethyl hydroxybenzotriazole **168** was made by treating the trifluoromethyl nitrobenzene **167** with hydrazine hydrate in ethanol, giving the desired HOBT **168** in 48% yield. The phosphonating reagent **169** was then made by reaction of methyl phosphonic dichloride with the HOBT **168** in the presence of pyridine. Due to the high reactivity of the phosphonating reagent **169** no purification was attempted and the material was used as a crude 0.125 M solution in dioxane.

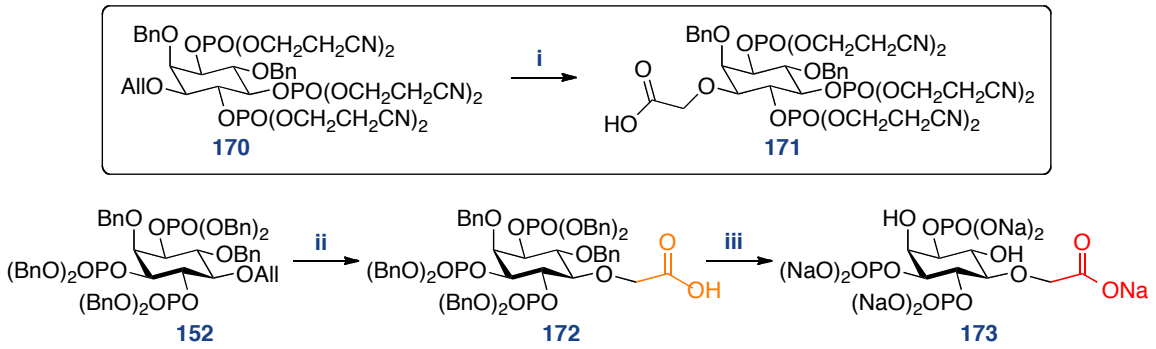
Alcohol **153** was dried over P_2O_5 for 18 h prior to reaction with phosphonating reagent **169**. This procedure ensured that as much water as possible was excluded from the reaction, thereby reducing any problematic side reactions. Dry pyridine was added to alcohol **153** and kept at 20 °C for the addition of phosphonating reagent **169**. Once the reagent **169** was added, consumption of the starting alcohol **153** was observed almost instantly. After 10 min, dry benzyl alcohol was added and the reaction stirred for a further 2 h. TLC analysis indicated a new major product had formed and mass spectrometry showed $[M+H]^+$ and $[M+H\cdot Py]^+$ for the desired product. Unfortunately, as with the 5-position sulfate precursor **163**, the methylphosphonate **165** proved inherently unstable. Purification by silica gel chromatography led to degradation of the product, and thus **165** could not be obtained with the required purity to perform the global deprotection.

4.8. Synthesis of Carboxylate 173

The synthesis of analogues using the 5-position alcohol as a handle for modification proved moderately successful. However, problems experienced with the stability of the precursor compounds have so far limited the utility of this approach. With work on-going regarding elaboration of alcohol **153**, our attention was drawn towards alternative approaches towards 5-position derivatives.

The precursor to alcohol **153** is the 5-position allyl protected ether **152**. It was postulated that the double bond of the allyl protecting group might be able to

undergo some interesting chemistry to attain 5-position derivatives that might be difficult to obtain *via* alcohol **153**. The presence of the double bond presents numerous possibilities that are not easily available from the alcohol such as, Grubbs cross metathesis, epoxide formation, halogenation, oxidative or reductive cleavage, and dihydroxylation. A literature search was conducted to find if reactions of this sort had been previously employed on inositol substrates. This search highlighted one paper by Potter *et al.* in which oxidative cleavage of the allylic double bond to give the carboxylic acid was achieved in the presence of three cyanoethyl protected phosphates in good yield (**scheme 4.6**).[110] It was hoped that using similar conditions would yield the carboxylic acid without affecting the benzyl protected phosphates.



Scheme 4.6. Published synthesis of carboxylic acid **171** from allylic ether.[110] *Reagents and conditions*: **i.** $RuCl_3 \cdot H_2O$, $NaIO_4$, CCl_4, MeCN, H_2O, RT, 64% yield. Synthesis of carboxylate 5-position Ins(1,3,4,5)P_4 derivative **173**. *Reagents and conditions*: **ii.** $RuCl_3 \cdot H_2O$, $NaIO_4$, CCl_4, MeCN, H_2O, RT, 40% yield; **iii.** $NaHCO_3$, Pd black, H_2, ^{t}BuOH/H_2O, RT, 85% yield.

The allylic ether **152** was treated with sodium periodate and ruthenium(III) chloride at room temperature. The reaction proceeded smoothly with consumption of starting material observed after 2 h. Work-up and isolation of the major product gave an oil that was contaminated with ruthenium by-products. ^{1}H and ^{31}P NMR analysis showed that the desired carboxylate **172** had formed (**scheme 4.6**); unfortunately repeated silica gel chromatography, reverse phase and ion exchange chromatography all failed to remove the black colouration from the ruthenium adducts. The compound also proved to be fairly unstable and ultimately decomposed during the various purification techniques attempted.

However, unpublished work conducted by Knight in Cardiff also experienced problems with ruthenium by-products. It was discovered that by conducting an oxidative wash using H_2O_2 solution during the aqueous work-up procedure they could oxidise any remaining ruthenium(III) to ruthenium(IV) oxide. The insoluble ruthenium(IV) oxide could be removed through filtration, giving sub PPM levels of ruthenium in their desired product. Consequently the oxidative cleavage of allylic ether **152** was repeated which again smoothly proceeded to completion, giving the desired carboxylic acid **172**. An oxidative work-up procedure using a 15% aqueous solution of H_2O_2 was employed; after the copious effervescence subsided the phases were filtered through Celite. Isolation of the major product by column chromatography gave the desired carboxylic acid **172**, free from ruthenium impurities.

Having successfully isolated carboxylic acid **172**, global benzyl group deprotection was attempted under standard conditions in the presence of 7 equivalents of $NaHCO_3$. This reaction proceeded smoothly to give the desired carboxylate **173** as the sodium salt in 85% yield, without the need for further purification.

4.9. Summary

A robust synthesis of the enantiomerically pure 5-position alcohol trisphosphate **153** has been developed. The utility of this approach was justified by the successful synthesis of two 5-position modified Ins(1,3,4,5)P_4 derivatives, methyl phosphate ester **157** and sulfamate **160** (**fig. 4.4**). However, the number of analogues produced was limited due to the instability of the final compound precursors for a number of potential 5-position Ins(1,3,4,5)P_4 derivatives.

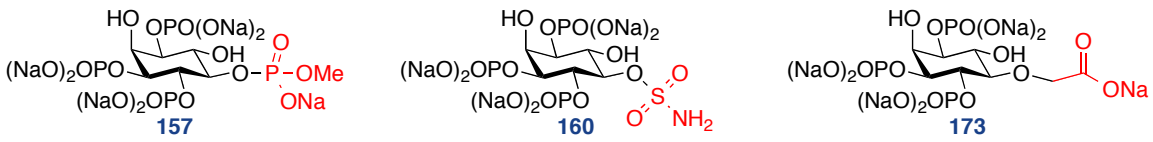


Figure 4.4. Summary of final comound 5-position modified Ins(1,3,4,5)P_4 derivatives

An alternative approach was employed, making use of the chemistry available from reactions with the 5-position allyl ether **152**. Oxidative cleavage of the allylic ether double bond enabled the successful synthesis of the enantiomerically pure 5-position carboxylate Ins(1,3,4,5)P_4 derivative **173** (**fig. 4.4**).

The three new 5-position derivatives **157**, **160** and **173** were submitted to biological analysis to assess their binding affinity for both the PBK and GRP1 PH domains.

5. Results and Discussion Part 3: The synthesis of 1-position modified Ins(1,3,4,5)P_4 analogues

5.1. Synthetic targets

Having successfully synthesised a collection of novel 5-position modified Ins(1,3,4,5)P_4 analogues, our attention was drawn towards expanding the number of 1-position modified Ins(1,3,4,5)P_4 analogues (**fig. 5.1**).

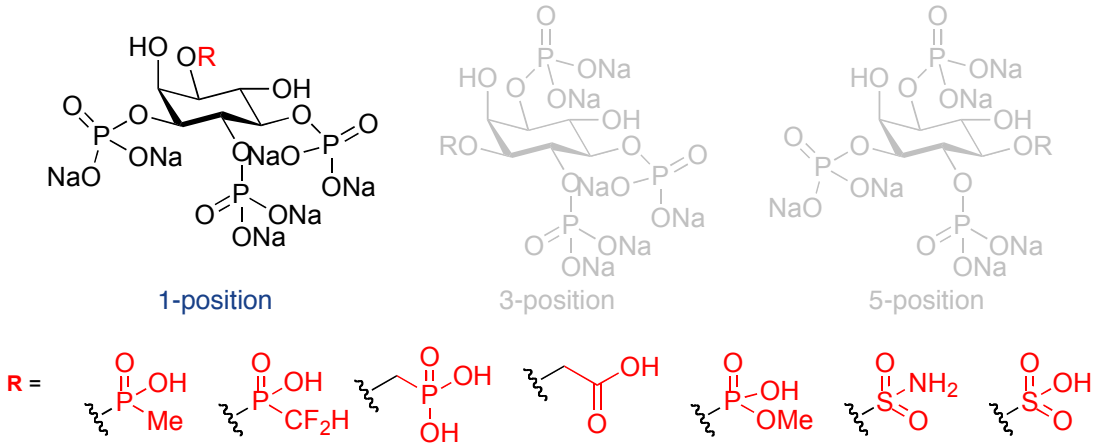
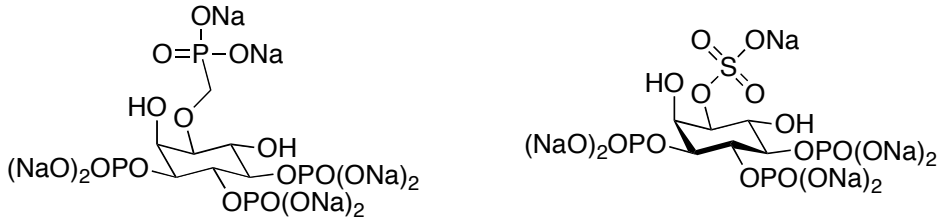


Figure 5.1. 1-Position modified targets.

Work conducted within the group by Nemeth,[31] successfully synthesised two novel 1-position modified Ins(1,3,4,5)P_4 derivatives, the sulfate **175** and the racemic methylene phosphonate **174** (**fig. 5.2**). These compounds were tested for their binding affinity towards the PKB and GRP1 PH domains. Neither analogue was shown to possess any binding affinity towards the PKB PH domain, however, both modifications were well tolerated within the GRP1 PH domain.



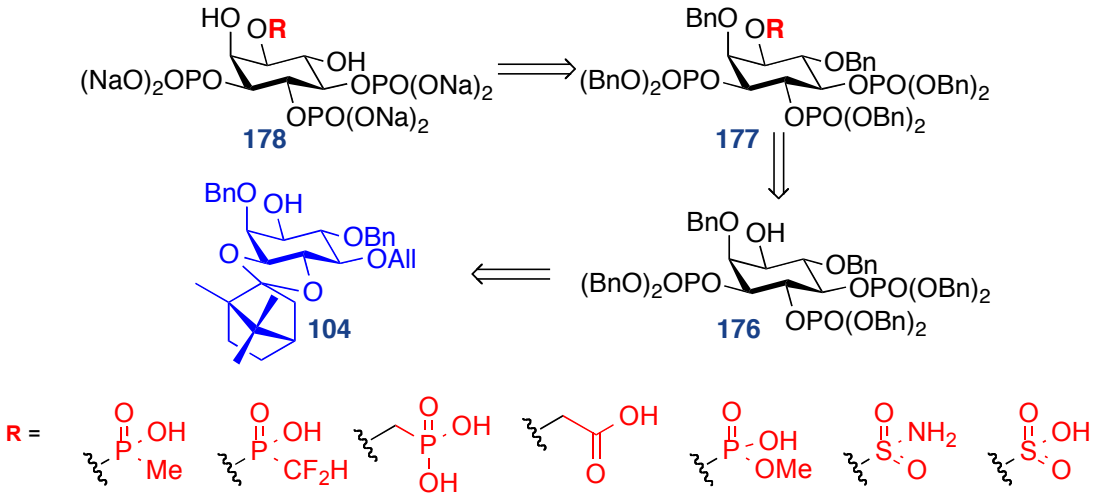
rac-JND137 (174) D-JNE38 (175)

Figure 5.2. 1-Position modified Ins(1,3,4,5)P_4 analogues synthesised within the group.[31]

In the first instance, it was desirable to resynthesise the methylene phosphonate **174** in enantiomerically pure form. This compound would be re-tested to assess whether any weak binding could be observed with the PKB-PH domain, as it is expected that in the enantiomerically pure form the compounds should be twice as potent, that is assuming that the L-enantiomer always shows no binding affinity. The sulfate **175** and methylene phosphonate **174**, showed almost equivalent binding affinity for the GRP1 PH domain. It is, therefore, desirable to synthesise a greater range of 1-position derivatives, to assess the impact of the different phosphate isosteres towards GRP1 and PKB PH domain binding.

5.2. Retrosynthesis

A general retrosynthesis of the enantiopure 1-position derivatives of Ins(1,3,4,5)P_4 **178**, is represented in **scheme 5.1**. The strategy towards 1-position derivatives was the same general approach that was used successfully for the synthesis of 5-position modified Ins(1,3,4,5)P_4 derivatives. In this approach, the final compounds **178,** are obtained by a global debenzylation of the appropriate precursor compounds **177**, which in-turn, are synthesised from the trisphosphate **176**.

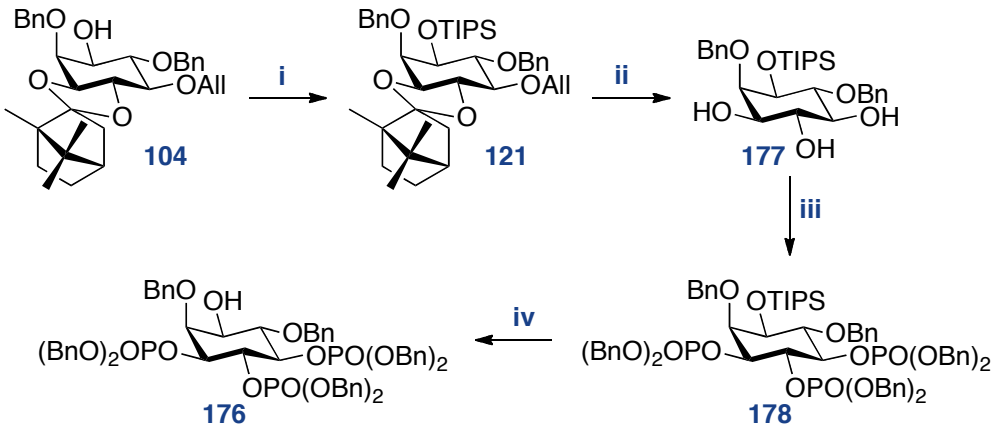


Scheme 5.1. A general retrosynthesis for 1-position modified ins(1,3,4,5)P_4 derivatives.

The desired alcohol trisphosphate **176** can be obtained from the enantiomerically pure alcohol **104** using the reliable synthetic route established by Nemeth.[31]

5.3. Synthesis of Alcohol trisphosphate 176

Commencing from the enantiomerically pure alcohol **104**, the 1-position hydroxyl was protected using TIPSOTf and Et_3N as opposed to TIPSOTf and 2,6-lutidine, which was utilised in the racemic synthesis of alcohol **176**. The modified conditions gave the TIPS ether **121** in 87% yield. In the racemic synthesis of alcohol **176**, triol **177** was made *via* sequential deprotection of intermediate **121**. Firstly methanolysis of the camphor acetal gave diol **105**; then methanolysis of the vinyl ether of **105**, formed through isomerisation of the allylic ether with Wilkinson's catalyst, gave the desired triol **177**. As both these deprotections utilise acid methanolysis it was postulated that both removal of the camphor acetal and the allylic ether could be accomplished in one pot.



Scheme 5.2. Synthesis of enantiopure alcohol **176**. *Reagents and conditions*: **i.** TIPSOTF, Et_3N, CH_2Cl_2, RT, 87% yield; **ii.** $PdCl_2$, MeOH, RT, 81% yield; **iii.** (a) bis(Benzyloxy)-*N*,*N*-diisopropylamino phosphine, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78°C 86% yield; **iv.** TBAF, THF, RT, 81% yield.

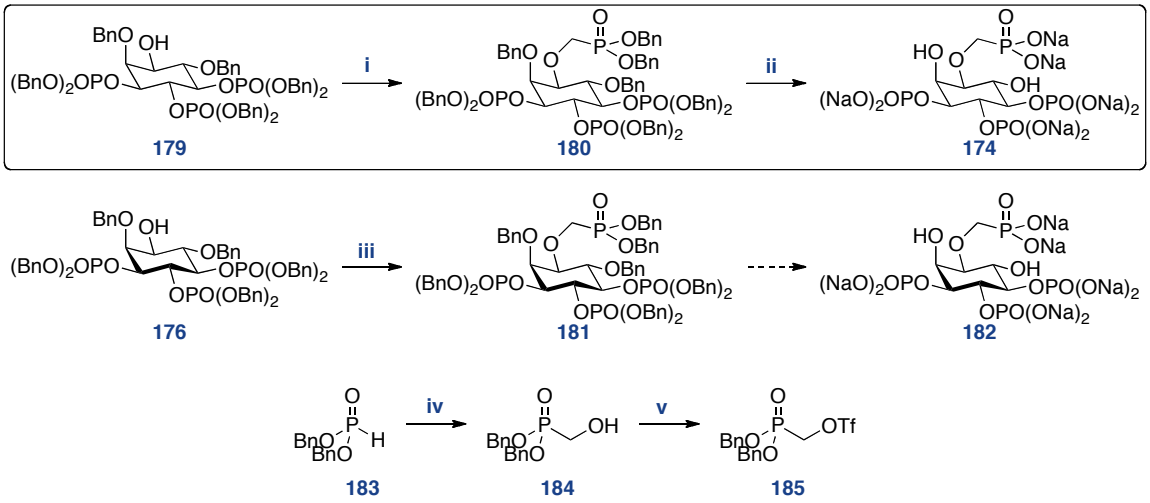
A solution of intermediate **121** in ethanol was treated with Hünig's base and Wilkinson's catalyst and heated under reflux. These conditions effected the isomerisation of allylic ether **121** to the vinyl ether within 3 h, as adjudged by ^{1}H NMR analysis. After replacing the reaction solvent with methanol, the reaction was treated with 4-TsOH. Acid-catalysed methanolysis of both the vinyl ether and the camphor acetal was indeed accomplished; with the desired triol **177** isolated in 37% yield. However, the low yield of the two simultaneous deprotections was not optimal, therefore, the $Pd(PPh_3)_4$ catalysed conditions for the removal of allyl groups published by Kusama and co-workers,[100] (successfully employed for the removal of allylic ether from diol **139** in chapter 3 giving PMB-triol **106** in high yield) were investigated. It was hoped that the same conditions might enhance the one-pot hydrolysis of **121** to give triol **177** in improved yield. Therefore, a solution of intermediate **121** in glacial acetic acid at 80 °C, was treated with $Pd(PPh_3)_4$. These conditions completed the desired transformation within 3 h, however, work-up and purification gave the triol in only 32% yield. In addition, ^{1}H and ^{31}P NMR analysis showed that some triphenylphosphine impurities were present. The triphenylphosphine impurities were a problem during the synthesis of the 5-position alcohol **153**, described in chapter 3, the answer to this problem was to use $PdCl_2$ for the allyl removal. Therefore, a solution of intermediate **121** in methanol was treated with $PdCl_2$ at

room temperature. The reaction proceeded smoothly, going to completion within 5 h; after work-up and simple purification the desired triol **177** was isolated in 81% yield (**scheme 5.1**).

Having developed a more robust and shorter synthesis of triol **177**, the remaining synthetic steps were accomplished in accordance with the route developed by Nemeth. Thus, triol **177** was reacted with excess bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** and catalytic 1*H*-tetrazole in CH_2Cl_2 at room temperature for 18 h. The crude reaction mixture was oxidised with *m*CPBA at -78 °C and upon work-up and chromatographic purification the desired trisphosphate **178** was isolated in 86% yield (**scheme 5.1**). The TIPS protected trisphosphate **178** was treated with TBAF in THF to affect the deprotection, affording alcohol **176** in 81% yield (**scheme 5.1**).

5.4. Attempted synthesis of methylene phosphonate 182

The initial aim of this synthesis was to repeat the synthesis of **174** in enantiomerically pure form, using the previously established chemistry. The methodology used involved reacting the dibenzyl phosphonomethyl triflate **185** with alcohol **179** in the presence of sodium hydride. These conditions gave the desired product, after exhaustive chromatography, in 24% yield. In this reaction, the use of sodium hydride was unavoidable, and as a result one can presume migration of the phosphates did occur, which might account for the poor overall yield of the reaction.



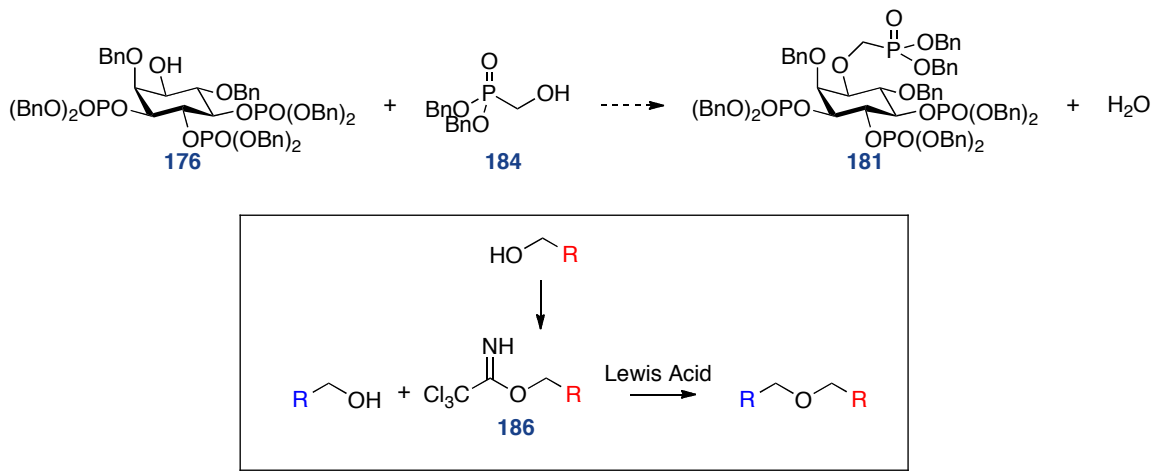
Scheme 5.2. Synthesis of racemic methylenephosphonate Ins(1,3,4,5)P_4 analogue **174**. *Reagents and conditions*: **i.** Triflate **185**, NaH, THF, -78 °C to RT, 24% yield; **ii.** $NaHCO_3$, Pd black, H_2, $^tBuOH/H_2O$, RT, 93% yield. Attempted synthesis of enantiomerically pure methylenephosphonate **182**. *Reagents and conditions*: **iii.** Triflate **185**, NaH, THF, -78 °C to RT. Synthesis of dibenzyl phosphonomethyl triflate **185**. *Reagents and Conditions*: **iv.** Paraformaldehyde, Et_3N, 130 °C, 25% yield; **v.** Trifluoromethanesulfonic anhydride, 2,6-lutidine, CH_2Cl_2, 75% yield.

The dibenzyl(hydroxymethyl)phosphonate **184** was synthesised according to the literature procedure,[111] by heating dibenzyl phosphite with paraformaldehyde and triethylamine at 130 °C for 20 min (**scheme 5.2**). After silica gel chromatographic purification, dibenzyl(hydroxymethyl)phosphonate **184** was isolated in 24% yield. Despite the poor yield, enough material was made in order to synthesise the required dibenzyl phosphonomethyl triflate **185**. This synthesis was accomplished using the literature procedure,[112] in which a solution of dibenzyl(hydroxymethyl)phosphonate **184** at -78 °C was treated with trifluromethane sulfonic anhydride and 2,6-lutidine. After work-up and isolation, the desired triflate was obtained in 75% yield (**scheme 5.2**).

Having made the required triflate, the same conditions used by Nemeth to make the racemic methylenephosphonate **180** were employed on the enantiomerically pure alcohol **176**. This reaction involved adding sodium hydride to a cooled solution of alcohol **176** and excess triflate **185**. It was hoped that having the triflate in excess and keeping the reaction cold, would reduce the chance of intermolecular phosphate migration, *via* attack of the alkoxide on a phosphate of

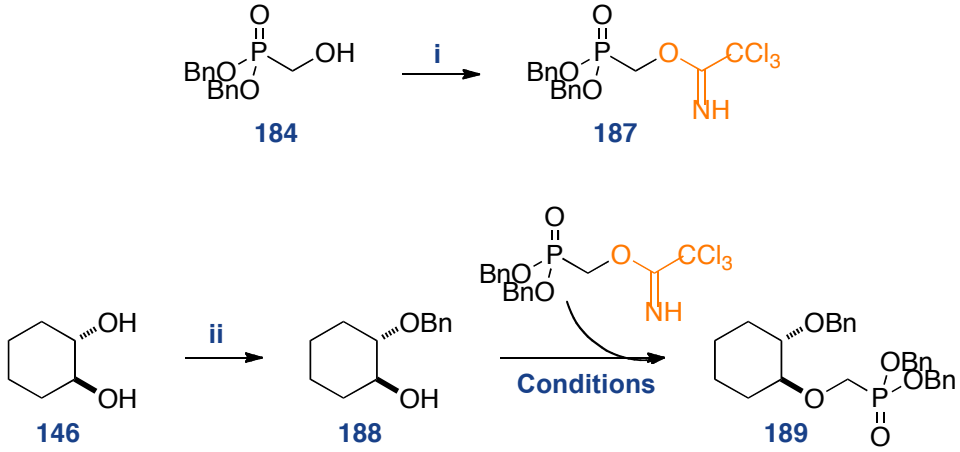
another molecule. However, as was found with the racemic synthesis, in order for the reaction to progress the mixture needed to warm to room temperature. Upon being allowed to slowly warm to room temperature, complete consumption of the starting material was observed within 2 h. After work-up the major product was isolated by silica gel column chromatography. ^{1}H and ^{31}P NMR analysis showed that the desired methylenephosphonate had indeed formed, however, a significant proportion of phosphate migration has also occurred. Repeated attempts to purify the compound *via* silica gel column chromatography, failed to separate the regioisomers and so the required purity for the global deprotection was not achieved.

Having been unsuccessful in repeating the methylenephosphonate synthesis using the established chemistry, alternative methods were considered. The fundamental problem with the reaction is that, in order to condense alcohol **176** with alcohol **184** to give ether **181** (**scheme 5.3**), alcohol **184** requires activation by formation of triflate **185**, and alcohol **176** requires activation *via* the alkoxide, which promotes phosphate migration. We therefore require milder conditions that avoid alkoxide formation. A common method to avoid activation of an alcohol by alkoxide formation is to activate the donor alcohol as a highly reactive leaving group. This activation is often achieved by the formation of the trichloroacetimidate (TCA) **186**, then reacting this with the desired alcohol *via* Lewis acid activation (**scheme 5.3**).



Scheme 5.3. Mild ether formation *via* use of trichloroacetimidate **186**.

It was desirable to investigate whether the trichloroacetimidate of alcohol **184**, would be reactive enough to undergo substitution without activation of alcohol **176** *via* the alkoxide. Hence, a study was conducted using model system whereby trichloroacetimidate **187** was synthesised and tested for reactivity against the simple model alcohol **188** with a range of Lewis acids.



Scheme 5.4. Model system for the mild synthesis of the methylenephosphonate **189**. *Reagents and conditions*: **i.** Trichloroacetonitrile, DBU, CH_2Cl_2, 0 °C to RT, 58% yield. Synthesis of model alcohol **189**. *Reagents and conditions*: **ii.** NaH, BnBr, DMF, 0 °C to RT, 48% yield.

The model alcohol acceptor **188** was synthesised in one step from *trans*-cyclohexane-1,2-diol by alkylation with benzyl bromide and sodium hydride. This reaction gave the model alcohol **188** in 48% yield. The trichloroacetimidate **187** was synthesised using standard conditions, whereby a solution of alcohol **184** in

dry CH_2Cl_2 at 0 °C was treated with trichloroacetonitrile and DBU. This reaction furnished the desired TCA-reagent in 58% yield. Having successfully synthesised the required reagents for the study, the trichloroacetimidate reactions were setup in parallel to test both a range of Lewis acid activators and some different reaction solvents reaction solvents. The results of the study are summarised in **table 5.1**.

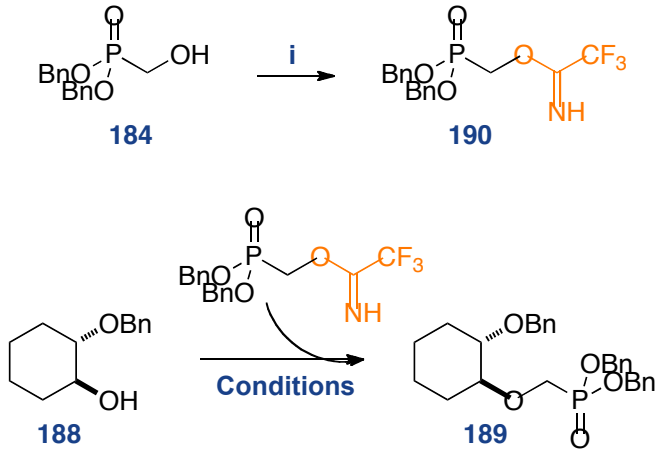
Entry	Lewis Acid	Solvent	Temperature	Reaction Observation
1	TMSOTf	THF	RT	Complex mixture, solvent polymerised
2	$BF_3{\cdot}Et_2O$	THF	RT	Complex mixture, solvent polymerised
3	$SnCl_4$	THF	RT	Complex mixture, solvent polymerised
4	$AgBF_4$	THF	RT	Complex mixture, some TCA hydrolysis product
5	TMSOTf	CH_2Cl_2	RT	No reaction after several days, starting material present
6	$BF_3{\cdot}Et_2O$	CH_2Cl_2	RT	Complex mixture of products
7	$SnCl_4$	CH_2Cl_2	RT	No reaction after several days, starting material present
8	$AgBF_4$	CH_2Cl_2	RT	No reaction after several days, starting material present

Table 5.1. Summary of conditions used, for the reaction of TCA-reagent **187** with model alcohol acceptor **188**.

When designing the experiment the choice of solvent was based on the requirement that it needed to be a polar aprotic solvent; DMF was avoided in the study as it was thought that Lewis acid coordination with the carbonyl of DMF might lead to undesirable side reactions. Therefore, THF and CH_2Cl_2 were chosen as reaction solvents. For each test reaction a solution of alcohol **188** in the desired solvent, was treated with TCA reagent **187**, followed by 0.1 molar equivalents of the appropriate Lewis acid. The reactions were stirred at room temperature and monitored by both TLC analysis and mass spectrometry. It quickly became apparent that THF was a poor choice of reaction solvent, as polymerisation of the solvent was observed, forming a thick gel that stalled all stirring (entries 1-3, **table 5.1**). Presumably, the Lewis acids were coordinating with the lone pair on the oxygen of the THF ring activating it towards nucleophilic attack from another THF ring. All the reactions using THF as a solvent formed

complex mixtures, no potential products were observed by either TLC or mass spectrometry.

Switching the solvent to CH_2Cl_2 resolved the problem of solvent polymerisation, however, TMSOTf, $SnCl_4$, and $AgBF_4$ (entries 5,7 and 8, **table 5.1**) failed to promote any reaction with the alcohol acceptor even after several days and with extra Lewis acid equivalents added. Ultimately, $[M+H]^+$ for the hydrolysed TCA-reagent and $[M+H]^+$ for the starting alcohol were the only discernable mass ions observed in the mass spectrum of the reaction mixture. $BF_3{\cdot}Et_2O$ (entry 6, **table 5.1**) saw rapid consumption of starting material, unfortunately, many products were observed by TLC analysis and mass spectrometry showed none of the desired mass ions. It became apparent that the trichloroacetimidate was too stable and that the secondary alcohol was too poor a nucleophile to react, even with the Lewis acid activated donor. This result brought us to postulate alternative stratagies for increasing the reactivity of the methylene phosphonate donor.



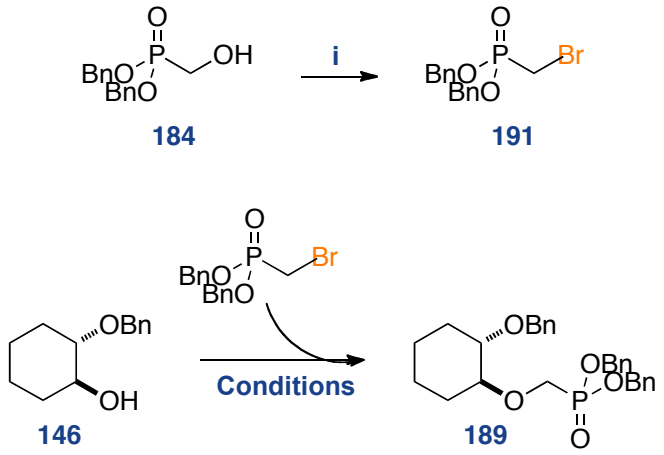
Scheme 5.5. Model system for the mild synthesis of methylenephosphonate **189**. *Reagents and conditions*: **i.** (a) Trifluoroacetamide, DMSO, oxalyl chloride, Et_3N, CH_2Cl_2, -78 °C, (b) alcohol **184**, DBU, CH_2Cl_2, -78 °C to RT, 40% yield.

A literature search found a report describing the use of trifluoroacetimidates (TFA) as an alternative to trichloroacetimidates.[113,114] The CF_3 unit has greater electronegativity and is thus, better able to stabilise the negative charge upon cleavage of the C-O bond, making it a better leaving group and a more reactive

species, often reacting *via* Brønsted acid catalysis rather than Lewis acid catalysis. It was hoped that the trifluoroacetimidate **190** would, therefore, be reactive enough to undergo our desired transformation. To synthesise the trifluoroacetimidate **190**, the literature contained similar methods to the synthesis of TCA reagents, utilising trifluoroacetonitrile as opposed to trichloroacetonitrile. The drawbacks to using trifluoroacetonitrile are that it is relatively expensive and is a highly toxic gas that requires a complicated setup. However, another method described in the literature makes use of trifluoroacetamide.[114] This much cheaper starting material is converted to trifluoroacetonitrile, *in situ*, *via* a Swern-type oxidation, which then reacts with the desired alcohol to give the required trifluoroacetimidate. Thus, to a solution of trifluoroacetamide in CH_2Cl_2 at -78 °C was added DMSO followed by oxalyl chloride and triethylamine. The reaction was stirred at -78 °C for 40 min before the addition of alcohol **184** as a solution in CH_2Cl_2. After purification by flash silica gel column chromatography, the desired trifluoroacetimidate **190** was isolated in 40% yield. Having successfully isolated the TFA reagent **190**, the coupling was attempted using a literature procedure. A solution of model alcohol **188** in CH_2Cl_2 was treated with TFA reagent **190** and pyridinium 4-toluenesulfonate (PPTS) at room temperature. The reaction was monitored by mass spectrometry and TLC analysis, but after several days only starting materials remained and no sign of a new product was observed. Eventually, hydrolysis of the TFA reagent back to alcohol **184** was observed. The reaction was repeated using TMSOTf as opposed to PPTS, to see if Lewis acid catalysis would promote the reaction, but as before, only starting material was observed after several days.

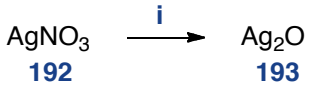
Both the TCA and TFA reagents were unreactive towards the secondary alcohol model compound **188**. One can tentatively explain this lack of reactivity when looking at the possible mechanism. During the transition state there is nothing to stabilise the developing positive charge on the methylene carbon. The electron withdrawing dibenzylphosphonate is α to the methylene carbon, thus formation of positive charge at this position is unfavourable.

Having failed to effectively activate the oxygen of alcohol **184** as a good leaving group, one more approach was considered before concentrating on other phosphate isosteres. It has been shown that alkyl bromides can undergo substitution with alcohols at room temperature in the presence of silver(I) oxide,[115] without the need of strong base to activate the alcohol. For this reason, the bromide **191** was also made for testing with the model alcohol **146** (**scheme 5.6**).



Scheme 5.6. Model system for the mild synthesis of methylenephosphonate **189**. *Reagents and conditions*: **i.** PPh_3, CBr_4, Et_2O, RT, 29% yield. **Conditions: (a)** Silver(I) oxide, DMF, RT; or **(b)** Silver(I) oxide (freshly prepared), DMF, RT.

The bromide **191** was accessed *via* an Appel-like reaction,[116] taking a solution of alcohol **184** in diethylether and treating this with carbon tetrabromide and triphenylphosphine gave the bromide **191** in 29% yield. Using conditions described in the literature for a similar process, a solution of model alcohol **146** and bromide **191** in DMF was treated with silver(I) oxide. The reaction was stirred at room temperature and monitored by TLC and mass spectrometry analysis. Due to the light sensitive nature of silver(I) oxide, the reaction was kept in the dark. However, as before no reaction was observed even with further additions of bromide **191** and silver(I) oxide and with heating. It was noted in the literature that reactions of this sort often require freshly prepared silver(I) oxide, because it's intrinsic instability towards light.

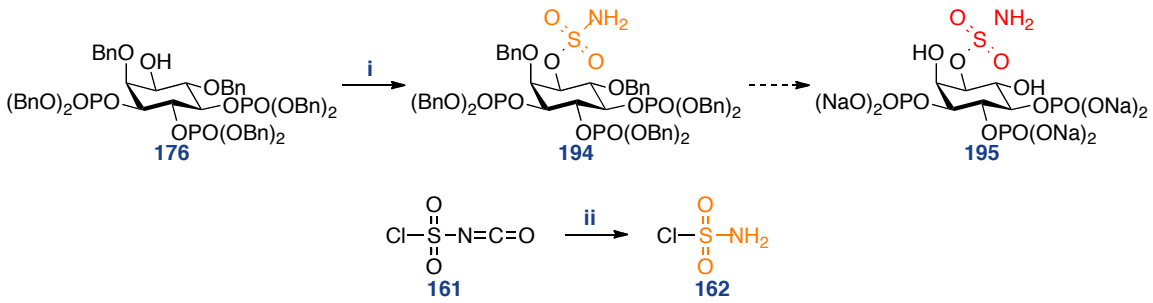


Scheme 5.7. Synthesis of silver(I) oxide. *Reagents and conditions*: **i.** NaOH, H_2O, RT, 48% yield.

Silver(I) oxide was prepared by treating silver(I) nitrate with sodium hydroxide solution (**scheme 5.7**), the silver(I) oxide precipitate was isolated by filtration. The coupling reaction was repeated using the freshly prepared silver(I) oxide. However, this modification had no effect on the outcome of the reaction. No reaction occurred after several days and with heating.

5.5. Attempted synthesis of sulfamate 195

The synthesis of the enantiomerically pure methylene phosphonate **182** using the established chemistry was unsuccessful, and the development of a new milder approach for its synthesis did not produce the desired results. Consequently, our focus shifted towards expanding the range of 1-position Ins(1,3,4,5)P_4 derivatives using chemistry that has been established for the installation of phosphate isosteres on similar derivatives. The successful synthesis of a novel 5-position modified sulfamate derivative **160** was described in chapter 3. It was hoped that using the same chemistry on the 1-position alcohol **176** would yield the sulfamate precursor **194** which would in-turn give the novel 1-position modified sulfamate final compound **195** (**scheme 5.8**).

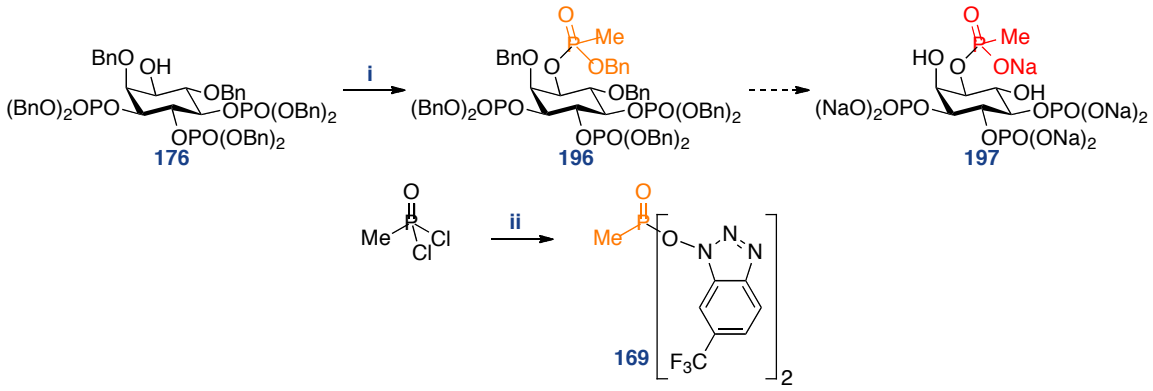


Scheme 5.8. Attempted synthesis of sulfamate **195**. *Reagents and conditions*: **i.** Sulfamoyl chloride **162**, DMA, 0 °C → RT. Synthesis of sulfamoyl chloride **162**. *Reagents and conditions*: **ii.** CSI, formic acid, CH_2Cl_2, 0 °C, used as a 2.50 M solution in CH_2Cl_2.

Alcohol **176** was treated with sulfamoyl chloride in the manner described in chapter 4. As previously observed, the reaction proceeded smoothly to completion within 18 h. After work-up and purification ^{1}H NMR analysis showed that the desired sulfamate had indeed formed, with $[M+NH_4{\cdot}MeCN]^+$ for the required mass ion being observed in the mass spectrum. However, the ^{31}P NMR revealed other phosphorus peaks shouldering the three major peaks, which is indicative of migration. A higher resolution ^{1}H NMR spectrum showed small peaks in the inositol region that had previously been over-lapped by the major inositol signals, which again provides evidence that phosphate migration had occurred. Further attempts to purify the product, using column chromatography, failed to give any improvement in the quality of the material.

5.6. Attempted synthesis of methylphosphonate 197

The installation of the methylphosphonate isosteric phosphate replacement has been conducted successfully within the group for the synthesis of Ins(1,4,5)P_3 derivatives.[49] Installation of the methylphosphonate onto the 5-position alcohol as described in chapter 3 was unsuccessful. It was hoped, however, that because the 1-position neighbours the axial 2-position benzyl ether, the less sterically demanding environment might lend itself to easier substitution and more stable precursors.



Scheme 5.9. Attempted synthesis of methylphosphonate **197**. *Reagents and conditions*: **i.** (a) Phosphonating reagent **169**, Py, 20 °C, 10 min, (b) BnOH; Synthesis of phosphonating reagent **197**. *Reagents and conditions*: **ii.** HOBT **168**, Py, dioxane, used crude as a 0.125 M solution in dioxane.

A solution of alcohol **176** in dry pyridine was treated with the phosphonating reagent **169**, as before, the starting material was consumed within 15 min. After this time the reaction was quenched with the addition of dry benzyl alcohol in order to displace the second HOBT unit, and form the desired benzyl protected methylphosphonate. A new major product was observed by TLC analysis and mass spectrometry found $[M+NH_4 \cdot MeCN]^+$ corresponding to the desired methylphosphonate **196**. However, the compound was found to be highly unstable towards column chromatography, giving rise to products corresponding both to the starting material and to highly polar impurities corresponding to debenzylation products. Attempts to purify the compound using neutral alumina and reverse phase chromatography, both failed to give any separation of products, with the compound being particularly unstable with regard to reverse phase chromatography. As a result of the inherent instability towards standard purification techniques, the desired precursor compound **196** could not be synthesised with the required purity to conduct the global deprotection.

5.7. Synthesis of methylphosphate ester 198

A particularly interesting analogue to access was methylphosphate ester (**fig. 5.3**) 1-position derivative of Ins(1,3,4,5)P_4 **198**, as this compound resembles PtdIns(3,4,5)P_3, more than any of the previous 1-position modified Ins(1,3,4,5)P_4 derivatives. It was, therefore, expected that this compound might show high affinity for the PKB PH domain as well as be tolerated in the GRP1 PH domain.

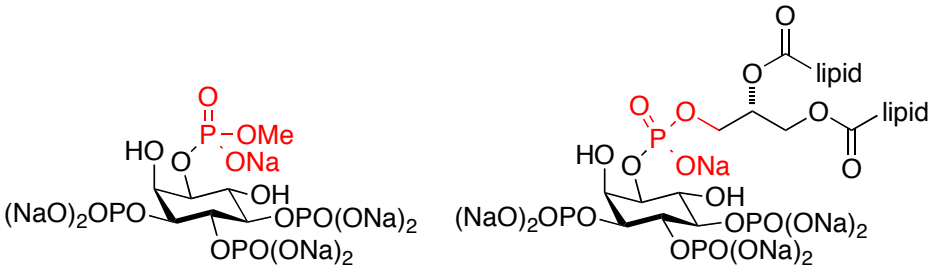
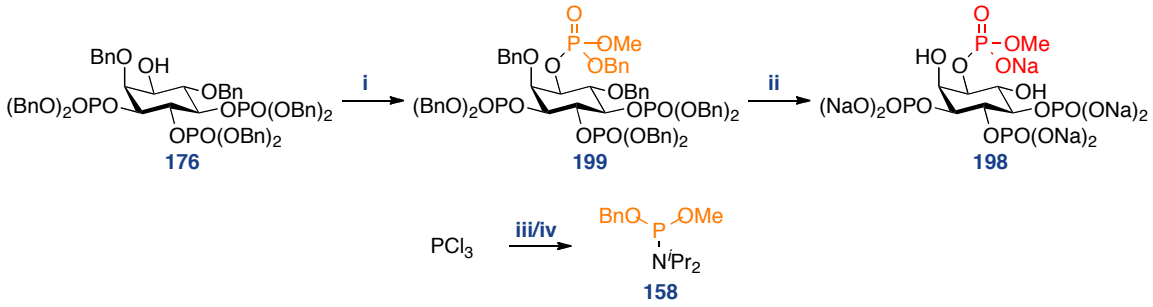


Figure 5.3. Desired methyl phosphate ester analogue **198** and PtdIns(3,4,5)P_3.

The methylphosphate ester isostere was successfully installed on the 5-position alcohol using phosphorus(III) chemistry, as described in chapter 4. It was hoped that by utilising these same mild phosphitylation conditions the 1-position methylphosphate ester precursor compound **199** could be obtained (**scheme 5.10**).



Scheme 5.10. Synthesis of methylphosphate ester Ins(1,3,4,5)P_4 derivative **198**. *Reagents and conditions*: **i.** (a) Phosphoramidite **158**, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78°C, 83% yield; **ii.** $NaHCO_3$, Pd black, H_2, ^{t}BuOH/H_2O, RT, quantitative yield. Synthesis of phosphoramidite **158**. *Reagents and conditions*: **iii.** Py, BnOH, diisopropylamine, Et_2O, 81% yield; **iv.** 1*H*-tetrazole, MeOH, CH_2Cl_2, 76% yield.

The racemic phosphoramidite **158** was made in two steps, as described in the previous chapter. A solution of alcohol **176** in CH_2Cl_2 was subsequently treated with excess phosphoramidite **158** in the presence of 1*H*-tetrazole. The reaction proceeded smoothly to completion within 18 h, as adjudged by TLC analysis. Mass spectrometry analysis showed $[M+NH_4{\cdot}MeCN]^+$ corresponding to the phosphorus(III) intermediate. *m*CPBA was added to the reaction mixture at -78 °C and then the mixture was allowed to warm to room temperature, after which time oxidation to the phosphorus(V) species was deemed complete by TLC analysis. Work-up and purification gave the desired methyl phosphate ester **199** in 83% yield as a 1.3:1 mixture of diastereomers as adjudged by ^{1}H NMR spectroscopy. Importantly, there was no sign of migration products and, gratifyingly, the intermediate appeared stable towards column chromatography. Having obtained the precursor **199** in a pure form the global deprotection of the benzyl protecting groups proceeded smoothly. The hydrogenolysis was conducted in the presence of $NaHCO_3$ to give, what is assumed to be, the

hepta-sodium salt. Isolation of the product gave the final compound **198** in quantitative yield without the need for further purification.

5.8. Summary

A synthetic route towards novel 1-position modified enantiomerically pure Ins(1,3,4,5)P_4 derivatives had previously been established within the group. This route involved the synthesis of enantiomerically pure alcohol **176** in five steps from the versatile alcohol **104**. An improvement to this synthesis has been made by the development of a one-pot synthesis of triol **177** from intermediate **104**. This modification shortened the synthetic route and provided a more reliable and higher yielding synthesis of alcohol **176**.

Attempts to expand the range of enantiomerically pure 1-position modified Ins(1,3,4,5)P_4 derivatives proved difficult. The initial attempt to synthesise the methylenephosphonate derivative **182** was unsuccessful due to significant migration of the phosphate esters. The migration was attributed to the use of the strong base, NaH. Model studies were conducted, in an effort to discover a milder method for the incorporation of the methylene phosphonate unit, these however proved unsuccessful. Migration was also observed during the attempted synthesis of the sulfamate analogue **195** and the methylphosphonate precursor **197** was found to be very unstable towards standard purification techniques.

Using the phosphorus(III) chemistry that was developed for the successful synthesis of the 5-position methylphosphate ester derivative **157** (chapter 4), it was possible to conduct the analogous reaction with the 1-position alcohol. This reaction gave the 1-position methylphosphate ester precursor **199** in excellent yield and purity. The presursor **199** subsequently underwent global deprotection to give the desired 1-position methylphosphate ester derivative **198**.

Having been successful in expanding the range of 1-position modified Ins(1,3,4,5)P_4 derivatives, compound **198** was subjected to biological analysis to assess its binding affinity for both the PBK and GRP1 PH domains.

6. Results and Discussion Part 4: Biological Analysis of Novel PtdIns(3,4,5)P_3 Analogues

6.1. Binding of PtdIns(3,4,5)P_3 Analogues to GRP1 PH domain

The biological evaluation of our novel PtdIns(3,4,5)P_3 analogues was conducted by our collaborators Professor Pete Downes and Dr Alex Gray at the College of Life Sciences, University of Dundee.

As described in chapter 1, previously unpublished biological analysis was conducted on the compounds synthesised by Nemeth (**fig. 6.1**).

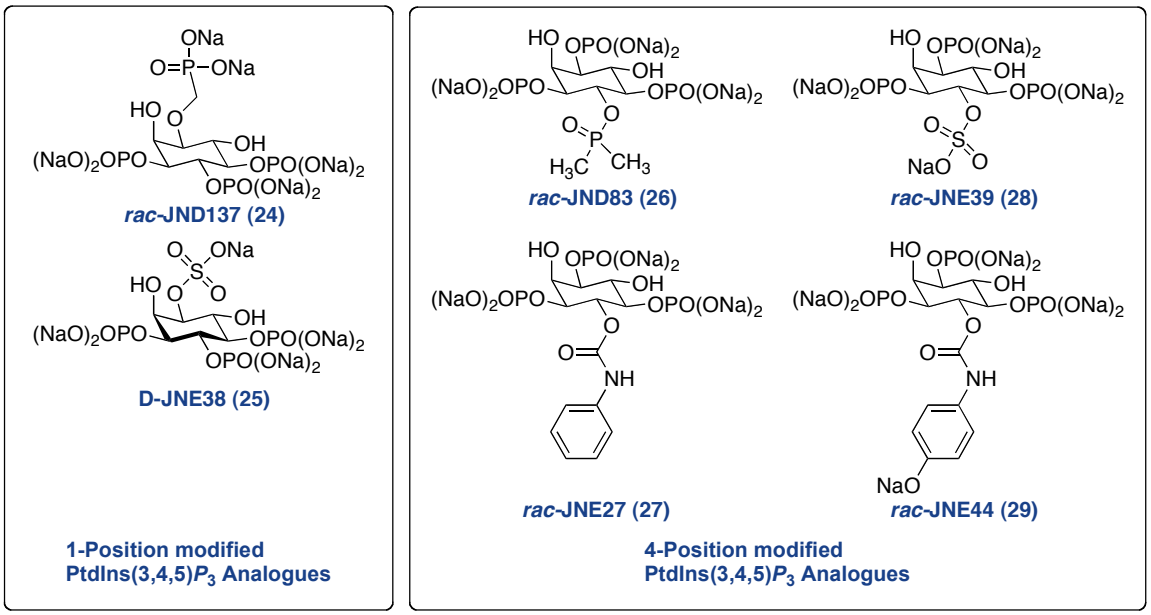


Figure 6.1. Compounds synthesised by Dr Joseph Nemeth for testing with GRP1 and PBK PH domains.

The compounds synthesised by Nemeth were tested for binding affinity towards both the GRP1 PH domain and the PKB PH domain. The results of these tests showed that both the 1-position modified PtdIns(3,4,5)P_3 analogues **24** and **25** bound to the GRP1 PH domain selectively over the PKB PH domain and that substitution of the 4-position phosphate was not tolerated by either PH domain containing protein.

6.2. Evaluation of PtdIns(3,4,5)P_3 Analogues for GRP1 PH Domain Binding

Having successfully synthesised a range of novel 5-position modified PtdIns(3,4,5)P_3 analogues and expanded on the range of 1-position modified PtdIns(3,4,5)P_3 analogues, these compounds were sent for biological evaluation to our collaborators in Dundee.

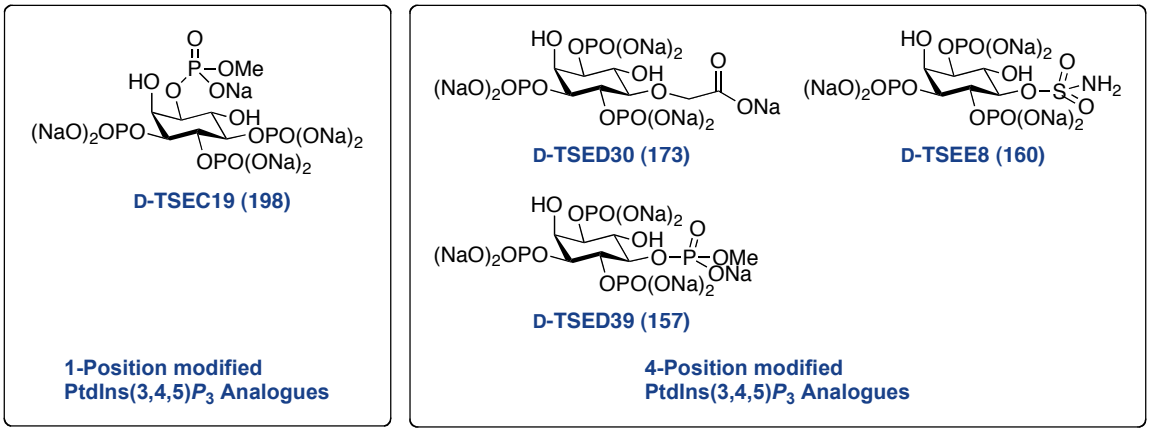


Figure 6.2. Novel PtdIns(3,4,5)P_3 analogues for testing with GRP1 and PBK PH domains.

The initial testing of these compounds was for binding affinity towards the GRP1 PH domain. The results are shown in **figure 6.3**.

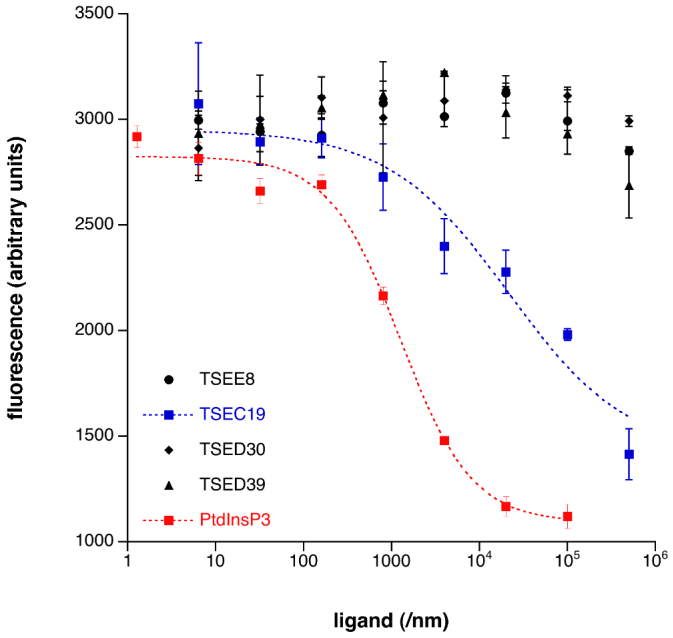


Figure 6.3. Graphical representation of the fluorescence quenching experiment, examining the binding affinity of compounds **157**, **160**, **173** and **198** for the GRP1 PH domain compared with PtdIns(3,4,5)P_3.

Figure 6.3 shows the relative binding affinities of each ligand with the GRP1 PH domain. The plot in red shows the natural ligand PtdIns(3,4,5)P_3 binds strongly at concentrations above 1 μM. All the 5-position modified PtdIns(3,4,5)P_3 analogues **157**, **160** and **173** (plotted in black) showed no binding affinity to the GRP1 PH domain up to concentrations of 1000 μM. The plot in blue shows that the 1-position modified methylphosphate ester analogue **198** possesses strong binding at concentrations above 10 μM. These results reinforce and complement the results obtained from the analysis of our previous 1-position analogues **24** and **25**, as both these analogues showed binding to the GRP1 PH domain with an equivalent affinity to that of **198**. It is interesting to see that modification at the 5-position, as with the 4-position modification, is not well tolerated for GRP1 PH domain binding, this indicates that both 4- and 5-position phosphates are crucial for binding. Additionally, it is interesting to note that the 1-position methylphosphate ester **198** shows a 10 fold reduction in binding compared to that of the natural PtdIns(3,4,5)P_3. The methylphosphate ester analogue **198** most closely resembles PtdIns(3,4,5)P_3 the difference being the lack of the

glycerol lipid diester unit, indicating that the glycerol diester moiety must contribute to the strength of PtdIns(3,4,5)P_3 binding.

6.3. Evaluation of PtdIns(3,4,5)P_3 Analogues for PKB PH Domain Binding

Having generated data for the GRP1 PH domain binding, testing was conducted for binding to the PKB PH domain. Our previous results showed that both the PKB and GRP1 PH domains did not tolerate modifications at both the 1- and 4-position phosphates.

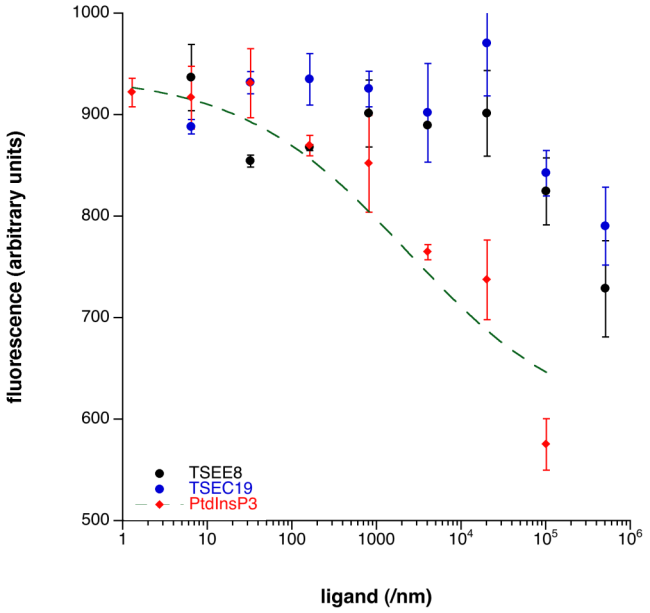


Figure 6.4. Graphical representation of the fluorescence quenching experiment, examining the binding affinity of compounds **198** and **160** for the PKB PH domain compared with PtdIns(3,4,5)P_3.

Figure 6.4 shows that the natural ligand PtdIns(3,4,5)P_3 (plot in red) binds with the PKB PH domain strongly above concentrations of 1 μM. The plot in blue represents the 1-position modified methylphosphate ester **198** this shows binding to the PKB PH domain at concentrations above 10 μM. This is the first analogue that we have produced that shows binding to the PKB PH domain, although it may not be surprising that this analogue does bind as **198** very closely resembles PtdIns(3,4,5)P_3. As with the binding of methylphosphate **198** to the GRP1 PH domain, there is a significant reduction in affinity compared to that of

PtdIns(3,4,5)P_3, indicating that again the glycerol diester moiety contributes to the binding strength in the PKB PH domain as well as to binding within the GRP1 PH domain. The plot in black represents the 5-position modified sulfamate analogue **160**, interestingly this compound also shows binding to the PKB PH domain at concentrations above 10 μM. The remaining 5-position analogues are yet to be tested for PKB PH domain binding affinity, nevertheless, the sulfamate present at this position is well tolerated, it can be hypothesised that the more acidic carboxylate and methylphosphate ester analogues might also show good binding with PKB PH domain, given the basic nature of the residues close to the 5-position phosphate of Ins(1,3,4,5)P_4 bound to the PKB PH domain. We have now shown that some of our analogues are able to discriminate between the GRP1 and PKB PH domains. The sulfamate **160** shows binding to PKB PH domain but not to the GRP1 PH domain, whereas both the sulfate **25** and methylene phosphonate **24** show binding to the GRP1 PH domain but not to the PKB PH domain. This mutual selectivity really highlights the potential for the development of PH domain selective inhibitors.

6.4. SAR of PtdIns(3,4,5)P_3 Analogues to GRP1 PH domain Binding

Having obtained biological data we can now use the published X-ray crystal structures of Ins(1,3,4,5)P_4 bound PKB PH domain and Ins(1,3,4,5)P_4 bound GRP1 PH domain to interpret our findings and start drawing up some structure activity relationship (SAR).

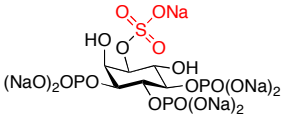
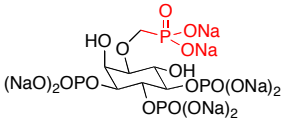
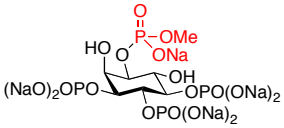
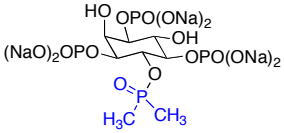
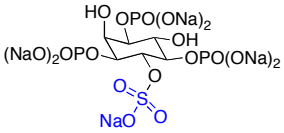
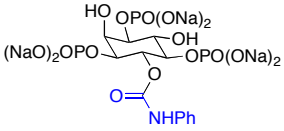
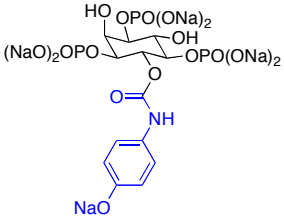
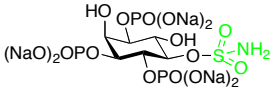
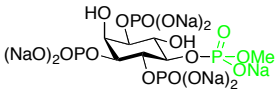
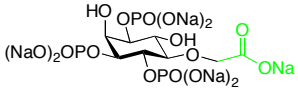
Position Modified	Compound	GRP1 PH Domain Binder	PKB PH Domain Binder
1-position modified		Yes (~0.1 μMol)	No
		Yes (~0.1 μMol)	No
		Yes (~0.1 μMol)	Yes (~10 μMol)
4-position modified		No	No
		No	No
		No	No
		No	No
5-position modified		No	Yes (~10 μMol)
		No	Not yet tested
		No	Not yet tested

Table 6.1. Summary of novel PtdIns(3,4,5)P_3 analogues and their biological activity.

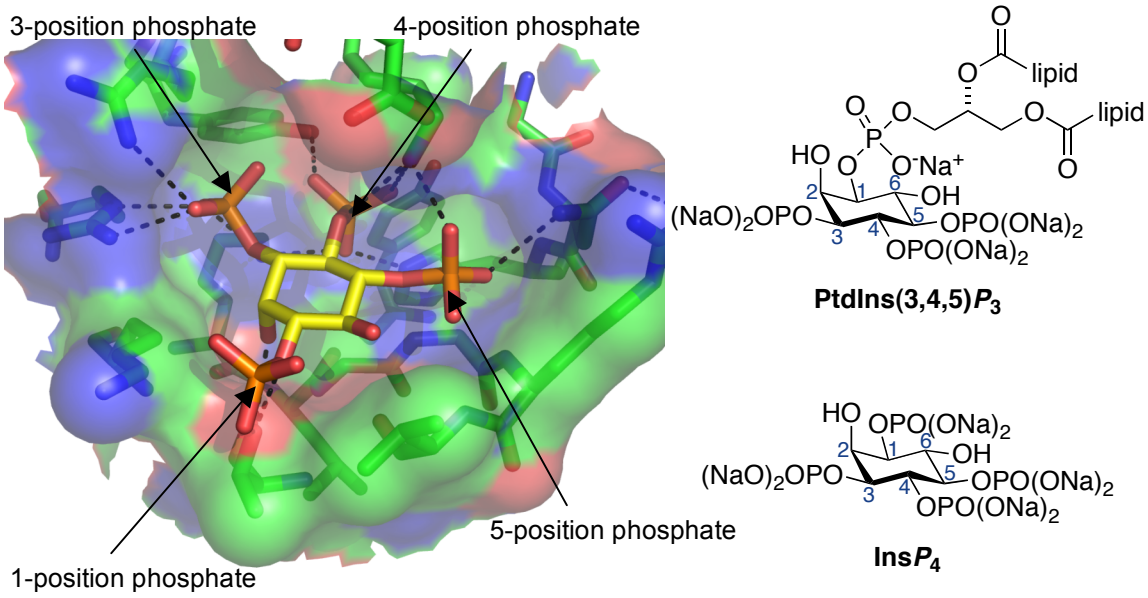


Figure 6.5. PyMOL representation of the X-ray crystal structure of Ins(1,3,4,5)P_4 bound GRP1 PH domain.[21] Expanded view of Ins(1,3,4,5)P_4 binding mode.

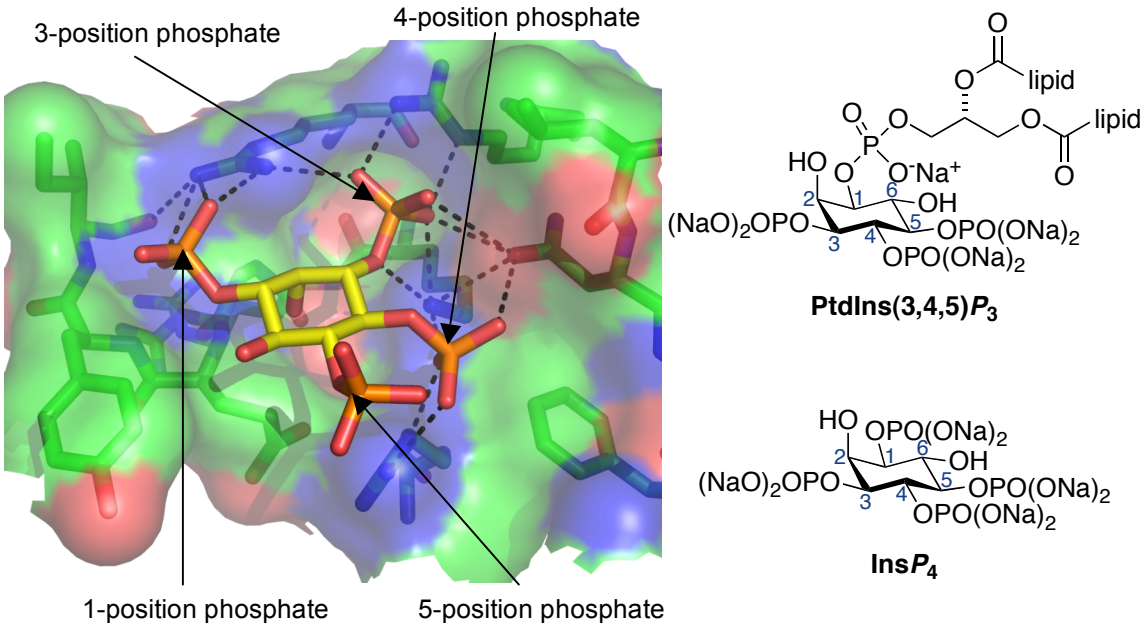
The X-ray crystal structure of Ins(1,3,4,5)P_4 bound GRP1 PH domain was obtained by Lambright *et al.*[21] From inspecting the binding of Ins(1,3,4,5)P_4 in the GRP1 PH domain (**fig. 6.5**), it can be seen that the 4-position phosphate is deeply embedded within the binding pocket, forming a complex hydrogen bonding network with several amino acids and at least two conserved water molecules. It is evident from our results that the 4-position phosphate is crucial for binding, as even with the highly acidic sulfate moiety, no affinity for the GRP1 PH domain is observed. Similarly, the 5-position phosphate forms multiple hydrogen bonds with several amino acids and conserved water molecules. One can see from the PyMOL representation (**fig. 6.5**) that basic residues surround the 5-position phosphate. Modification of the 5-position was not tolerated, despite replacing the phosphate with acidic functionality. Clearly the 4- and 5-position phosphates are equally important for GRP1 PH domain recognition. Interestingly a report by Lemmon *et al.*,[37] comparing the binding affinity of a variety of multiply phosphorylated *myo*-inositols showed that Ins(1,3,4,5)P_4 bound the GRP1 PH domain very strongly with a $K_D \approx 27$ nM, but that Ins(1,3,4)P_3 bound with an approximate $K_D \approx 2$ μM. Our 5-position modified derivatives showed no binding affinity at concentrations up to 1000 μM,

suggesting that our phosphate replacements were more detrimental to binding than having no phosphate present at all.

Modification of the 1-position phosphate of Ins(1,3,4,5)P_4 was well tolerated within the GRP1 PH domain. From the X-ray crystal structure (**fig. 6.5**), it can be seen that the 1-position phosphate is a lot more solvent exposed and only makes one hydrogen bonding interaction with Thr-280 on the β1/β2 loop, indicating that the 1-position is less important for GRP1 PH domain recognition, and thus tolerates modification well. It will be interesting to test 3-position modified derivatives, as the 3-position phosphate in the GRP1 PH domain makes an equivalent number of hydrogen bonding interactions as the 4- and 5-position phosphates. From this one can hypothesise that 3-position modifications are likely to not be well tolerated.

6.5. SAR of PtdIns(3,4,5)P_3 Analogues to PKB PH domain Binding

The X-ray crystal structure of Ins(1,3,4,5)P_4 bound PKBα PH domain was obtained by van Aalten and co workers (**fig. 6.6**).[38] Direct comparison of Ins(1,3,4,5)P_4 bound PKBα PH domain with the Ins(1,3,4,5)P_4 bound GRP1 PH domain, one can immediately see that the Ins(1,3,4,5)P_4 binds in the same mode, with the axial 2-position hydroxyl pointing down into the binding pocket.


3-position phosphate
4-position phosphate
1-position phosphate
5-position phosphate
lipid
lipid
HO
O
O
P
O
O
O

the phosphate. The methyl phosphate ester **198**, which showed binding the PKB PH domain, also contains two unsubstituted oxygen atoms at the methyl phosphate moiety, this is presumably why this modification was tolerated. However, the sulfate and methylene phosphonate modified derivatives, **25** and **24**, did not bind. These compounds most likely could not form the required orientation for making the comparable hydrogen bonds at variable loop 1 that the methyl phosphate ester **198** and Ins(1,3,4,5)P_4 can make. If one looks at the differences in binding of the 1-position phosphate in the PKB PH domain, compared with that of the 1-position phosphate in the GRP1 PH domain. The 1-position phosphate makes only one direct hydrogen bond with Thr280 on the β3-β4 loop of the GRP1 PH domain whereas in the PKB PH domain it makes five hydrogen bonding interactions with Tyr18, Ile19 and Arg23 on variable loop 1. This significant difference highlights the importance of the 1-position phosphate for PKB-PH domain binding as well as explaining the observed tolerance that the GRP1 PH domain has for 1-position modified derivatives.

The 5-position phosphate of Ins(1,3,4,5)P_4 makes no direct hydrogen bonding interactions with the PH domain of PKB (**fig. 6.6**), which explains why the 5-position modified sulfamate **160** showed binding, as no key binding interactions will have been disturbed upon the binding of **160**. Comparison of how the 5-position phosphate of Ins(1,3,4,5)P_4 interacts with the PH domains of both PKB and GRP1 reveals significant differences. One can see that the 5-phosphate makes five direct hydrogen bonds with residues in the GRP1 PH domain (**fig. 6.5**) compared to zero in the PKB PH domain (**fig. 6.6**). This observation highlights the importance of the 5-position phosphate for GRP1 PH domain binding and explains the selectivity observed between the two PH domains.

6.6. Summary

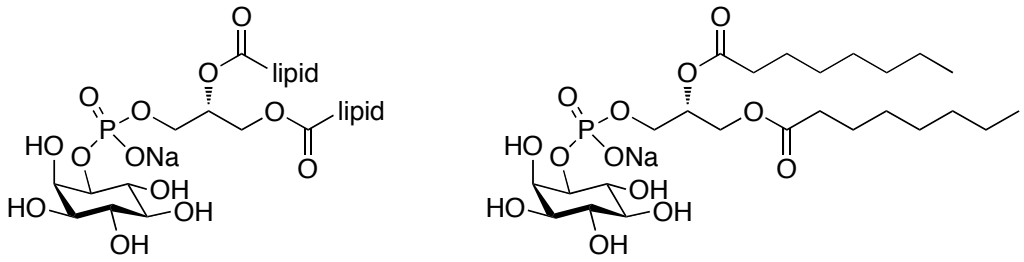
We have been able to develop a range of PtdIns(3,4,5)P_3 analogues and have tested them for binding to both the PKB PH and GRP1 PH domains. Our results have shown, that for binding to either PH domain, the 4-position phosphate appears crucial. As analogues bearing highly acidic phosphate isosteres at this

position showed no binding affinity. The 1-position phosphate appears to be less important for GRP1 PH domain binding compared to that of PKB PH domain binding, with all 1-position analogues binding to the GRP1 PH domain. The 5-position phosphate appears to be very important for GRP1 PH domain binding, but much less important for PKB PH domain binding. Due to these intrinsic differences within their PH domains, we have successfully synthesised mutually selective PH domain binders, with the 1-position sulfate **25** and methylene phosphonate **24** selectively binding the GRP1 PH domain over the PKB PH domain. But the 5-position sulfamate **160** binding to the PKB PH domain selectively, over the GRP1 PH domain.

7. Results and Discussion Part 5: The synthesis of Phosphatidylinositol

7.1. Synthetic Target

Although the primary aim of the project is the synthesis of PtdIns(3,4,5)P_3 analogues, another important inositol phosphate is phosphatidyl inositol (PtdIns) **13**. PtdIns is the biosynthetic precursor to phosphatidylinositol 3-phosphate, phosphatidylinositol 4-phosphate and phosphatidyl inositol 5-phosphate, which are subsequently transformed into the many and important multiply phosphorylated PtdInsP_ns, which play important roles in cellular signalling.



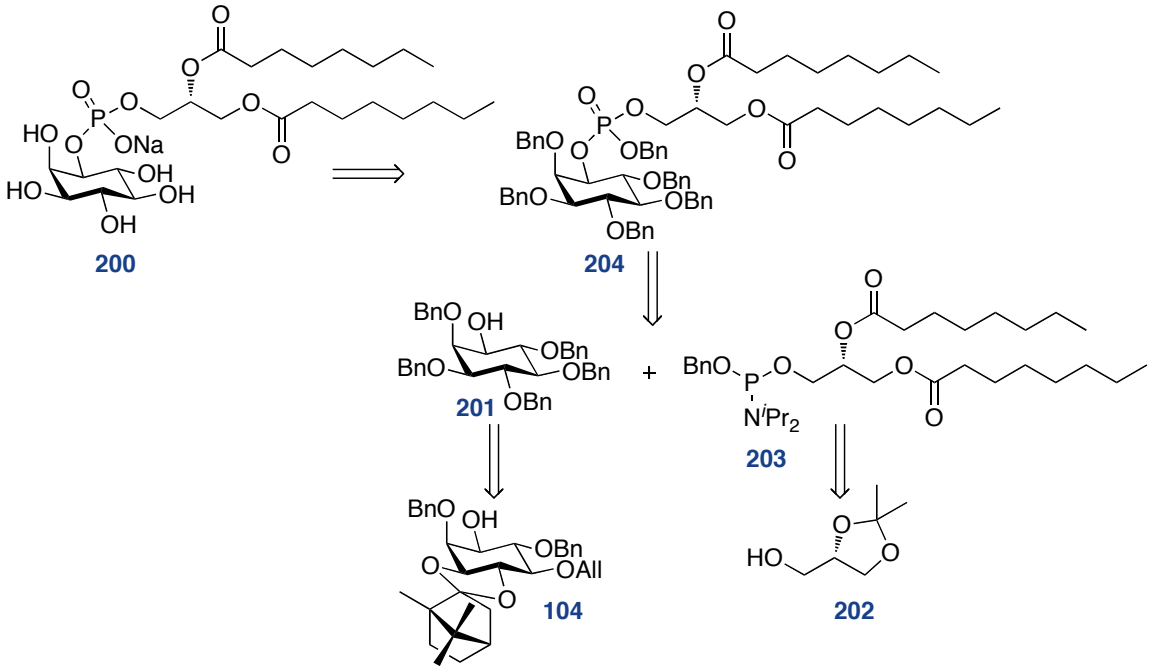
PtdIns (13) **Dioctanoyl PtdIns (200)**

Figure 7.1. A general structure of PtdIns derivatives **13** and the structure of target dioctanoyl PtdIns **300**.

The structure of the lipid chain in PtdIns isolated from natural sources (**13, fig. 7.1**) varies depending on the source. Many synthetic PtdIns also vary in the type of lipid chain used. We were interested in developing a robust synthesis of dioctanoyl PtdIns **200** (**fig. 7.1**); this has superior water solubility than other PtdIns derivatives, which has advantages when used for biological assays or in crystallisation studies.

7.2. Retrosynthesis

A general retrosynthesis for PtdIns **200** is represented in **scheme 7.1**. The retrosynthesis of PtdIns **200** follows a similar approach to the one used for the successful synthesis of 1- and 5-position Ins(1,3,4,5)P_4 derivatives, described in chapters 3 and 4.

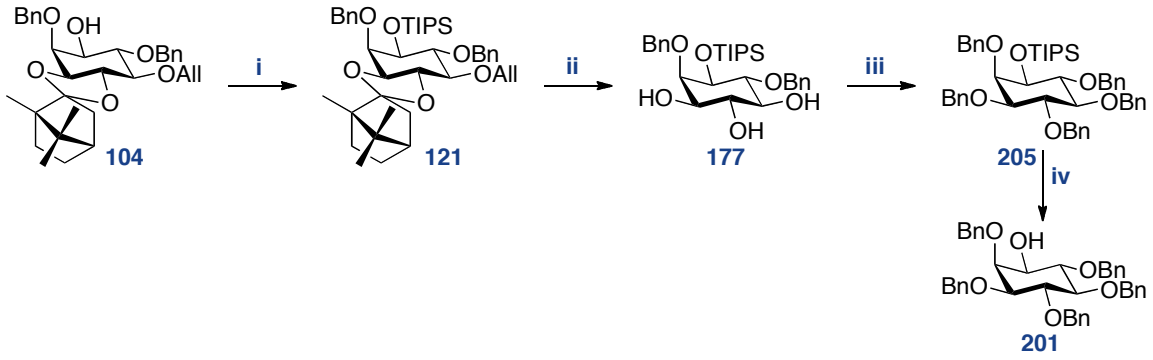


Scheme 7.1. General retrosynthesis for dioctanoyl PtdIns

The final compound **200** would be obtained from a fully protected precursor compound **204**, *via* a global deprotection step. The precursor compound **204** can be broken into two fragments, the inositol fragment **201** and the phospholipid fragment **203**. The coupling of the two fragments will be mediated *via* the reliable phosphorus(III) chemistry employed for the installation of the benzyl protected phosphate esters and the methyl phosphate esters. The inositol fragment **201** could hopefully be obtained reliably from the versatile enantiomerically pure intermediate **104**, and the lipid phosphoramidite **203** could be obtained from the enantiomerically pure (+)-1,2-*O*-isopropylidene glycerol **202**. As only a single position around the inositol ring is substituted, the key for the synthesis of insitol fragment **201** is to obtain the required 1-position orthogonality. It was envisioned that this would be accomplished using the same method as was utilised within the synthesis of 1-position modified Ins(1,3,4,5)P_4 derivatives described in chapter 4.

7.3. Synthesis of Inositol Fragment 201

The synthesis of the required inositol fragment **201** commences from the enantiomerically pure alcohol **104**.[89] The synthesis of alcohol **104** was described in chapter 3.

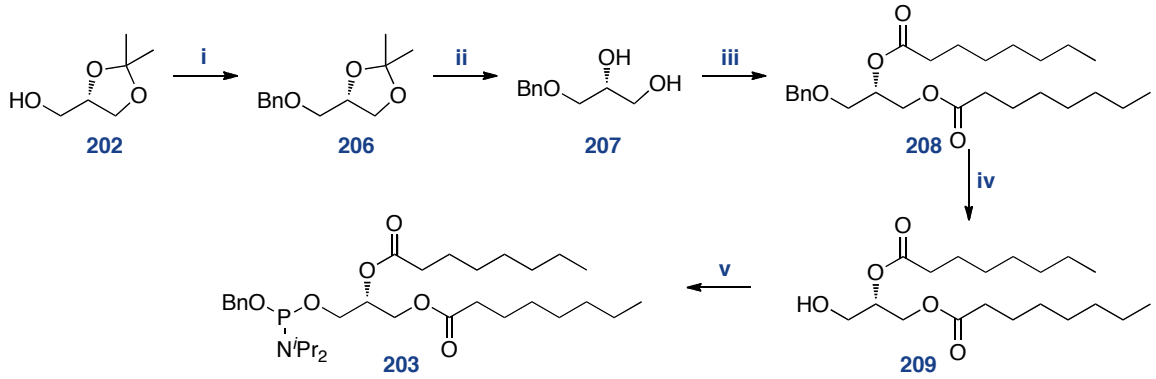


Scheme 5.2. Synthesis of enantiomerically pure alcohol **201**. *Reagents and conditions*: **i.** TIPSOTF, Et_3N, CH_2Cl_2, RT, 87% yield; **ii.** $PdCl_2$, MeOH, RT, 81% yield; **iii.** BnBr, NaH, DMF, 0 °C → RT, 68% yield; **iv.** TBAF, THF, RT, 88% yield.

TIPS protection of the 1-position hydroxyl group of alcohol **104** was conducted using the conditions optimised previously. This reaction gave TIPS ether **121**, which has the required othogonality at the 1-position. Other syntheses have orthogonally protected the 1-position alcohol with a PMB ether, however, work within the group has found PMB ethers to be sensitive to certain acidic conditions. We have found the TIPS ether can be added and removed very reliably in high yields, but is also stable to a multitude of conditions. Both the camphor acetal and the allylic ether were removed using the one-pot $PdCl_2$-mediated procedure, to give the vicinal triol **177**. The triol **177** was treated with NaH and benzyl bromide to afford the perbenzylated intermediate **205** in 68% yield. Reasonably forcing conditions were required for the benzylation, with an excess of both benzyl bromide and NaH required to push the reaction to completion. The steric demands imposed by the benzylation, of three neighbouring hydroxyls, likely accounts for the moderate yield experienced. The synthesis of the desired inositol fragment was completed by removal of the TIPS ether using TBAF in THF; this reaction gave the desired alcohol **201** in 88% yield.

7.4. Synthesis of Lipid Fragment 203

The synthesis of the lipid fragment **203** was completed using conditions similar to those that have been previously reported.[117-120] Nemeth completed the initial 3 steps of the synthesis, whereby the (+)-1,2-*O*-isopropylidene glycerol **202** was protected as the benzyl ether **206**, acidic methanolysis subsequently gave the diol **207**, which was then reacted with octanoyl chloride in the presence of catalytic DMAP, to give the diester **208**.

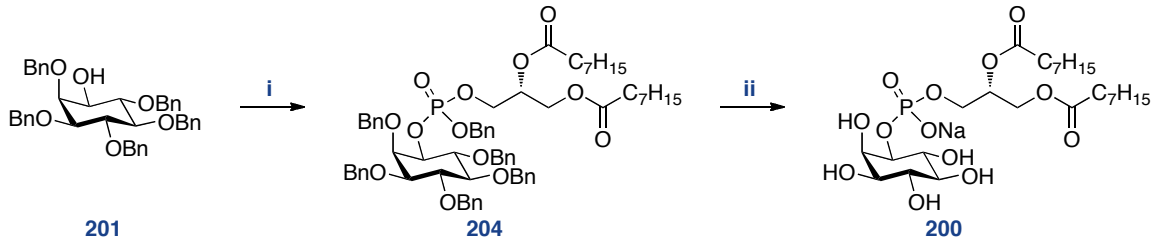


Scheme 7.3. Synthesis of enantiopure lipid fragment **203**. *Reagents and conditions*: **i.** NaH, BnBr, DMF, RT, 92% yield; **ii.** conc. HCl, MeOH, reflux, 96% yield; **iii.** DMAP, pyridine, octanoyl chloride, RT, 92% yield; **iv.** $Pd(OH)_2$, H_2, THF, RT, 87% yield; **v.** Benzyloxybis(*N*,*N*-diisopropylamino)phosphine **141**, 1*H*-tetrazole, CH_2Cl_2, RT, 71% yield.

The benzyl group of the protected diester **208** was then removed by $Pd(OH)_2$ catalysed hydrogenolysis, to give the desired alcohol **209** in 87% yield. Reaction of alcohol **209** with benzyloxybis(*N*,*N*-diisopropylamino)phosphine **209** and 1*H*-tetrazole in CH_2Cl_2, gave the desired phosphoramidite **203**, in 71% yield. This reaction completed the synthesis of the lipid fragment for coupling to the inositol fragment.

7.5. Synthesis of Dioctanoyl PtdIns

Having successfully synthesised the desired inositol fragment **201** and the desired lipid fragment **203**, we could proceed with the coupling to give the precursor compound **204**.



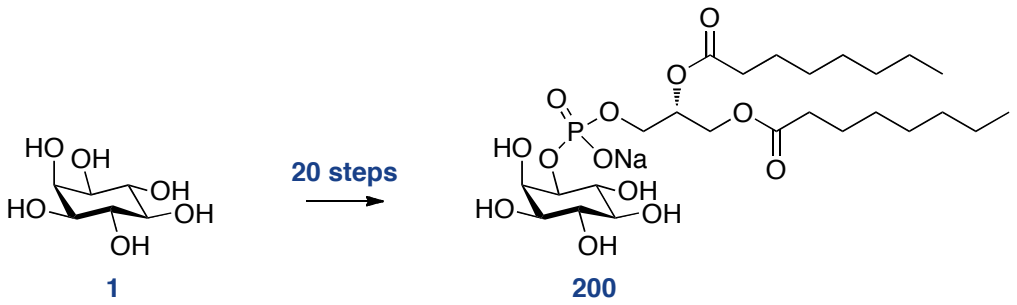
Scheme 7.4. Synthesis of enantiopure dioctanoyl PtdIns **200**. *Reagents and conditions*: **i.** (a) phosphoramidite **203**, 1*H*-tetrazole, CH_2Cl_2, RT, (b) 3-Chloroperoxybenzoic acid, -78 °C, 52% yield; **ii.** $NaHCO_3$, Pd black, H_2, $^tBuOH/H_2O$, RT, 64% yield.

Thus, a solution of alcohol **201** in CH_2Cl_2 was treated with the lipid phosphoramidite **203** in the presence of 1*H*-tetrazole at room temperature. The reaction proceeded smoothly; however, further equivalents of the phosphoramidite were required after 18 h. Stirring the reaction mixture for an additional 3 h saw the complete consumption of starting material as adjudged by TLC analysis. It is assumed the the phosphorus(III) intermediate had formed at this point. Therefore, the reaction was cooled to -78 °C and treated with *m*CPBA in order to oxidise the phosphorus(III) to phosphorus(V). The purification required exhaustive silica gel column chromatography in order to give the desired precursor **204** in 52% yield. The troublesome purification was attributed to impurities arising from the lipid phosphoramidite. The highly non-polar nature of both **204** and the lipid impurities made separation difficult, which accounts for the moderate isolated yield. Nevertheless, the precursor **204** was isolated in the required purity to proceed with the global deprotection. Palladium black-mediated hydrogenolysis proceeded smoothly and was conducted in the presence of one equivalent of $NaHCO_3$ to give the presumed sodium salt. Isolation of the product gave the final compound **200** in 64% yield, without the need for further purification.

7.6. Summary

The versatile enantiomerically pure alcohol **104** has allowed for the development of a very reliable and robust synthesis of dioctanoyl PtdIns. The TIPS ether is not frequently used in inositol chemistry, but has certainly proved it can be added and removed very reliably in high yields, and is stable to a number of conditions

that we utilise regularly. The use of the TIPS ether has allowed access to the useful intermediate alcohol **201** in high yield, which ultimately led to the successful synthesis of dioctanoyl PtdIns.



1 200

Scheme 7.5. The mono substitution of *meso* compound *myo*-inositol.[121]

What initially appears to be a rather trivial transformation (a mono substitution of the inositol ring with a single phospholipid [**scheme 7.5**]), has taken us 20 steps of synthesis, with a 13 step longest linear sequence and with an overall yield of 0.45%.[121] With the cell being able to accomplish the same transformation in one-step, this highlights the challenges faced when breaking the symmetry of a molecule like the *meso* compound *myo*-inositol and how important it is to develop robust methods for the desymmetrisation of compounds of this nature.

7.7. Future Work and Summary

This project has broadened the synthetic utility of the enantiomerically pure intermediate **104** by the successful synthesis of 1- and 5-position Ins(1,3,4,5)P_4 derivatives as well as the natural product PI. The approach used within the group, has been to develop a robust synthesis to an alcohol trisphosphate, of the respective position around the inositol ring that requires modification. Then using this alcohol as a handle for further reactions with a set of electrophiles, in order to incorporate the desired isosteric phosphate replacement. This approach has met with success, however certain drawbacks to the method are becoming apparent. The secondary alcohol, for instance, is not a particularly good nucleophile, a simple way to activate the alcohol is to form the alkoxide, however, we have found that this cannot be formed in the presence of phosphate esters, as it causes a significant amount of phosphate migration. Therefore, to avoid

activation of the alcohol, highly reactive electophiles are required. This propensity for migration under forcing conditions has begun to limit the number of phosphate isosteres that can be easily incorporated onto the inositol ring, this limitation is exemplified by the attempted synthesis of the methylene phosphonate moiety in chapter 5. It is, therefore, of interest to seek alternative approaches that reduce the susceptibility of phosphate migration and debenzylation pathways. One approach could be the use of dimethyl phosphates **211** (**fig. 7.2**), commonly used by Prestwich and co-workers **210** (**fig. 7.2**);[102] these would be less sterically strained, however, the global deprotection of these is not as mild as the hydrogenolysis of benzyl groups, and further purification steps for the final compounds are a likely requirement.

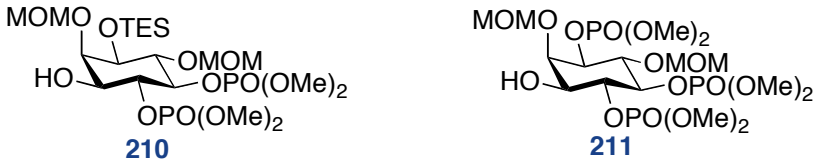
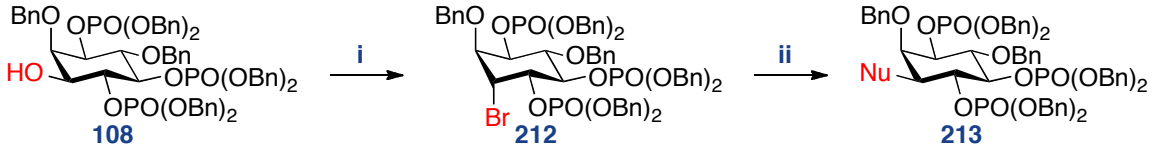


Figure 7.2. Prestwich compound **210** and methyl-protected trisphosphate **211** as a potential alternative to the sterically strained benzyl-protected trisphosphates.

Another approach would be to turn the inositol ring into the electrophile, and have the desired phosphate isostere behave as the attacking nucleophile. For example it may be possible to perform an Appel-type reaction on alcohol **108** to form the bromide **212** (**scheme 7.6**). In this way it may be possible to activate the desired nucleophile, with strong base for instance, in the absence of the benzyl-protected phosphates and then displace the bromide by the careful addition of the activated nucleophile. With no free hydroxyl group on the inositol ring migration cannot occur; also, limiting the exposure of the trisphosphate electrophile **212** to the activated nucleophile, might reduce the propensity of debenzylation because of the milder environment.



Scheme 7.6. Potential use of bromide **212**. *Appel-type reaction*: **i.** PPh_3, CBr_4; **ii.** Activated nucleophile.

With reliable access to 1-, 4- and 5-position derivatives of Ins(1,3,4,5)P_4 now developed, it is of continuing interest to research the synthesis of 3-position modified Ins(1,3,4,5)P_4 analogues. This will complete the substitution of all the phosphates of Ins(1,3,4,5)P_4 and allow us to plan a second generation of PH domain-targeted analogues, aimed at increasing both selectivity and binding affinity towards a particular PH domain.

8. Experimental Section

8.1. General Experimental

^{1}H NMR spectra were recorded on Bruker DPX250 (250 MHz); Bruker Avance 300 (300 MHz); Bruker Avance 400 (400 MHz); Bruker Avance II 400 (400 MHz); Bruker DRX500 (500 MHz); Bruker Avance 500 (500 MHz); or Bruker Avance III (500 MHz) using deuterochloroform (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as δH in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); ddt (doublet of doublet of triplets); sp (septet); or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software.

^{13}C NMR spectra were recorded on Bruker Avance 300 (75 MHz); Bruker Avance II 400 (100 MHz); or Bruker Avance III (125 MHz) spectrometers using the PENDANT or DEPT Q pulse sequences with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δC (TMS) = 0.00 ppm. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. ^{1}H and ^{13}C spectra were assigned using 2D NMR experiments including COSY, HSQC, HMBC, DEPT-135, HMQC, and DEPT Q.

^{31}P NMR spectra were recorded on Bruker Avance II 400 (162 MHz) or Bruker

DRX500 (202 MHz) spectrometers using broadband proton decoupling pulse sequences and deuterium internal lock. The chemical shift data for each signal are given as δP in units of parts per million (ppm) relative to 85% phosphoric acid as an external reference.

^{19}F NMR spectra were recorded on a Bruker Avance II 400 (282 MHz) using a broadband proton decoupling pulse sequence and deuterium internal lock. The chemical shift data for each signal are given as δF in units of parts per million (ppm). Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz.

Mass spectra were acquired on a VG platform spectrometer. For electron impact spectra, the instrument was operating at 70 eV. Chemical ionization spectra were obtained using isobutane as the ionising gas. Electrospray ionisation spectra were obtained on Micromass LCT; Micromass LCT Premier; and Bruker MicroTOF spectrometers, operating in positive or negative mode, from solutions of methanol, acetonitrile or water. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Melting points were determined using an Electrothermal 9100 melting point apparatus or Kofler hot stage microscope and are uncorrected.

Microanalyses were obtained on a Carlo Erber EA1110 analyser by the St Andrews University microanalysis service. Certain samples were submitted to the Elemental Analysis Service, London Metropolitan University, London.

Infrared Specta were obtained either as: **a** thin film on sodium chloride discs; **b** potassium bromide disc. The spectra were recorded on Perkin Elmer GX FT-IR or Bruker Tensor 27 spectrometers. Absorption maxima are reported in wavenumbers (cm^{-1}).

Specific Optical Rotations were measured using Perkin Elmer Model 241 and 341 polarimeters, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (T = ambient temperature in °C).

Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} aluminum-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 or 365 nm), or thermal development after dipping in one of: **a** ethanolic solution of phosphomolybdic acid (PMA); **b** aqueous solution of potassium permanganate, potassium carbonate and sodium hydroxide; **c** ethanolic solution of 4-anisaldehyde, sulfuric acid and acetic acid.

Flash Column chromatography was carried out on Apollo Scientific Ltd silica gel 40-63 micron or Merck silica gel 60 (240-400 mesh), eluting with solvents as supplied, under a positive pressure of compressed air (unless otherwise stated).

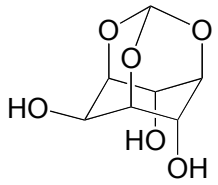
Anhydrous solvents were obtained under the following conditions: dry acetonitrile was distilled from calcium hydride in a recycling still; dry *N*,*N*-dimethylformamide was purchased from SigmaAldrich UK in a SureSeal™ bottle and used without further purification or was distilled under reduced pressure from activated 4 Å molecular sieves and stored over 4 Å molecular sieves under an N_2 atmosphere; dry dimethyl sulfoxide was pre-dried over activated alumina, then distilled from calcium hydride and stored over activated 4 Å molecular sieves under an N_2 atmosphere; anhydrous 1,4-dioxane was distilled from sodium and benzophenone in a recycling still and stored over activated 3 Å molecular sieves under an Ar atmosphere. Anhydrous dichloromethane, diethyl ether, toluene, hexane and tetrahydrofuran were obtained using a MBRAUN GmbH MB SPS-800 solvent purification system, where solvent was dried by passage through filter columns and dispensed under an atmosphere of N_2 or Ar gas.

Chemicals were purchased from Acros UK, Sigma Aldrich UK, Alfa Aesar UK, Fisher UK, Fluka UK, Fluorochem, Merck or TCI-Europe. All solvents and reagents were purified, when necessary, by standard techniques. Where appropriate and if not stated otherwise, all non aqueous reactions were performed in a flame-dried flask under an inert atmosphere of nitrogen or argon, using a double vacuum manifold with the inert gas passing through a bed of activated 4 Å molecular sieves and self indicating silica gel.

In vacuo refers to the use of a rotary evaporator attached to a diaphragm pump. Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to a mixture of hexane isomers and petroleum ether to the fraction boiling between 40-60 °C.

8.2. Synthesis of Enantiopure Intermediate 104

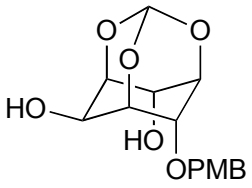
D-*myo*-Inositol 1,3,5-orthoformate 111



111

To a stirred solution of *myo*-inositol **1** (10 g, 55.56 mmol, 1 eq), in dry DMF (40 mL) under an atmosphere of nitrogen, was added 4-toluenesulfonic acid monohydrate (4.23 g, 22.22 mmol, 0.4 eq) followed by triethyl orthoformate (51.86 g, 46.21 mL, 277.80 mmol, 5 eq). The solution was heated to 100 °C and stirred overnight. The reaction was shown to be complete by TLC analysis and cooled to RT. The reaction was quenched by addition of solid sodium hydrogen carbonate (~5 g). The solid was removed by filtration, washed with methanol and discarded. The filtrate was concentrated under reduced pressure affording a pale yellow oil. The oil was dissolved in methanol (100 mL), and cooled to promote crystallisation of the product, which was isolated by filtration. The filtrate concentrated under vacuum yielding a pale yellow oil, which was adsorbed onto silica gel and the product purified by silica gel column chromatography, eluting first with CH_2Cl_2, then with MeOH and CH_2Cl_2 (5:95) and finally MeOH/CH_2Cl_2 (10:90). All product fractions were combined and the solvent removed under vacuum to yield D-*myo*-inositol 1,3,5-orthoformate **111** (6.66 g, 63% yield) as a colourless crystalline solid: R_f 0.5 (MeOH and $CHCl_3$ 20/80); mp 287-288 °C *dec*. (*from MeOH/CH_2Cl_2,* lit.[122] 300-302 °C sealed tube); δ_H (300 MHz, DMSO-D_6) 5.45 (2H, d, *J* 5.1, 2 × OH), 5.44 (1H, s, 3-H), 5.30 (1H, d, *J* 6.2, equatorial OH), 4.26 (2H, br s, 2 × inositol ring), 4.07-4.04 (1H, m, inositol ring), 4.01-3.97 (1H, m, inositol ring), 3.95-3.92 (2H, m, 2 × inositol ring). The data are in good agreement with literature values.[89,93]

(±)-D-4-*O*-(4-Methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate 112

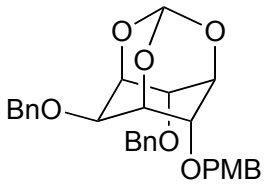


112

D-*myo*-Inositol 1,3,5-orthoformate **111** (21 g, 110.53 mmol, 1 eq) was dissolved in dry DMF (500 mL) under an atmosphere of nitrogen and cooled to 0 °C. With vigorous stirring, sodium hydride (60% dispersion in mineral oil, 4.86 g, 121.58 mmol, 1.1 eq) was added portionwise over a period of 1 h. The resulting suspension was allowed to warm to room temperature and stirred for a further 2 h, forming a thick white slurry. The slurry was cooled to 0 °C and 4-(methoxy)benzyl chloride (19.04 g, 16.50 mL, 121.58 mmol, 1.1 eq) was added dropwise with vigorous stirring. The reaction mixture was warmed to room temperature and stirred overnight. The reaction was shown to be complete by TLC analysis and so was quenched with water (50 mL). The volatile components were removed under vacuum yielding a thick yellow oil, which was partitioned between ethyl acetate (200 mL) and water (200 mL). The layers were separated and the aqueous phase extracted with ethyl acetate (3 × 100 mL). The combined organic components were washed with brine (200 mL) and dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum, yielding a yellow oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (20:80, 30:70, 40:60 and 50:50). The combined organic fractions were concentrated under vacuum yielding (±)-D-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **112** as a fine, colourless crystalline solid (30.75 g, 90% yield): R_f 0.35 (ethyl acetate and petroleum ether 1:1); mp 97-100 °C (*from ethyl acetate and petroleum ether,* lit.[123] 99-100 °C); δ_H (300 MHz, $CDCl_3$) 7.20-7.14 (2H, m, $OCH_2C_6H_4OCH_3$), 6.86-6.81 (2H, m, $OCH_2C_6H_4OCH_3$), 5.36 (1H, d, *J* 1.3, 3-H),

4.55 (1H, d, J_{AB} 11.4, OCH_AH$_B$), 4.50 (1H, d, J_{AB} 11.4, OCH$_A$$H_B$), 4.42-4.31 (2H, m, 2 × inositol ring), 4.20-4.11 (3H, m, 3 × inositol ring), 4.03-3.93 (1H, m, inositol ring), 3.75 (3H, s, OCH_3), 3.71 (1H, d, *J* 10.2, OH), 3.09 (1H, d, *J* 11.8, OH). The data are in good agreement with the literature values.[123]

(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate 113

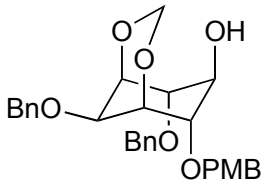


113

(±)-D-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **112** (4.00 g, 12.09 mmol, 1 eq) was dissolved in dry DMF (50 mL) under an atmosphere of nitrogen and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 1.30 g, 32.23 mmol, 2.5 eq) was added portionwise and stirred for 30 minutes at 0 °C followed by 1 h at RT forming a thick green gel. The gel was cooled to 0 °C and benzyl bromide (5.51 g, 3.83 mL, 32.23 mmol, 2.5 eq) was added dropwise, loosening the thick gel, the resulting slurry was allowed to warm to room temperature and stirred overnight. The reaction was shown to be complete by TLC analysis and the mixture was quenched with water (10 mL). The volatile components were removed under vacuum resulting in an oil, which was partitioned between ethyl acetate (20 mL) and water (20 mL). The layers were separated and the aqueous phase extracted with ethyl acetate (3 × 10 mL). The combined organic components were washed with brine (20 mL) and dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum yielding a yellow oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (20:80 then

30:70). The product fractions were combined and the solvent removed under vacuum to give (±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **113** (5.42 g, 91% yield) as a colourless oil: R_f 0.43 (ethyl acetate and petroleum ether 40:60); δ_H (300 MHz, $CDCl_3$) 7.42-7.20 (10H, m, 2 × Ph), 7.13 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 6.82 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 5.54 (1H, d, *J* 1.3, 3-H), 4.65 (2H, s, OCH_2Ph), 4.62 (1H, d, J_{AB} 11.6, OCH_AH_BPh), 4.55 (1H, d, $J_{A'B'}$ 11.2, $OCH_{A'}H_{B'}$-$C_6H_4OCH_3$), 4.48 (1H, d, J_{AB} 11.6, OCH_AH_BPh), 4.42 (1H, d, $J_{A'B'}$ 11.2, $OCH_{A'}H_{B'}$-$C_6H_4OCH_3$), 4.45-4.40 (1H, m, inositol ring), 4.38-4.26 (4H, m, inositol ring), 4.06-4.03 (1H, m, inositol ring), 3.81 (3H, s, OCH_3). The data are in good agreement with the literature values.[123]

(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-methylene 114

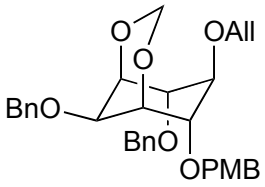


114

(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3,5-orthoformate **113** (14.23 g, 29.01 mmol, 1 eq) was dissolved in dry CH_2Cl_2 (140 mL) under a nitrogen atmosphere and cooled to 0 °C. With vigorous stirring, diisobutylaluminium hydride (72.52 mL of a 1 M solution in hexanes, 72.52 mmol, 2.5 eq) was added slowly dropwise maintaining the temperature at 0 °C (±5 °C). The reaction mixture was allowed to warm to RT slowly and react, affording a colourless solution overnight. TLC analysis indicated the full consumption of the starting material. The reaction mixture was cooled to 0 °C and the diisobutylaluminium hydride quenched slowly with the addition of water (2.9 mL, 0.04 mL per mmol of diisobutylaluminium hydride) (CAUTION: copious

effervescence and a slight exotherm is observed upon addition of water). The reaction mixture was treated with 15% (w/v) sodium hydroxide (aqueous) solution (2.9 mL, 0.04 mL per mmol of diisobutylaluminium hydride) followed by water (7.3 mL, 0.1 mL per mmol of diisobutylaluminium hydride). The solution was allowed to warm to RT and stirred for 1 h, yielding the aluminium salts as a colourless precipitate. The mixture was dried over magnesium sulfate and the solid components isolated *via* filtration. The filtrate was concentrated under vacuum to yield (±)-D-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-methylene **114** (13.18 g, 92% yield) as a thick colourless oil: R_f 0.34 (ethyl acetate and petroleum ether 40:60); δ_H (300 MHz, $CDCl_3$) 7.30-7.20 (10H, m, 2 × Ph), 7.12 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 6.75 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 5.48 (1H, d, *J* 4.9, 3-H), 4.60 (1H, d, J_{AB} 11.9, OCH_AH_B-Ph), 4.59 (1H, d, *J* 4.9, 3-H), 4.53 (2H, s, OCH_2-Ph), 4.53 (1H, d, $J_{A'B'}$ 9.5, $OCH_{A'}H_{B'}$-$C_6H_4OCH_3$), 4.49 (1H, d, $J_{A'B'}$ 9.5, $OCH_{A'}H_{B'}$-$C_6H_4OCH_3$), 4.43 (1H, d, J_{AB} 11.9, OCH_AH_B-Ph), 4.38-4.31 (2H, m, inositol ring), 4.22-4.20 (1H, m, inositol ring), 3.95-3.90 (2H, m, inositol ring), 3.87 (1H, d, *J* 10.2 inositol ring), 3.73 (3H, s, OCH_3), 2.90 (1H, d, *J* 10.2 OH). The data are in good agreement with the literature values.[123]

(±)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-methylene 115

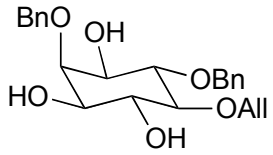


115

(±)-D-2,6-Bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-methylene **114** (13.18 g, 26.76 mmol, 1 eq) was dissolved in dry DMF (150 mL) under an atmosphere of nitrogen and cooled to 0 °C. With stirring, sodium hydride (1.6 g,

40.14 mmol, 1.5 eq) was added portionwise and the mixture allowed to warm to RT for 2 h before cooling to 0 °C. The resulting colourless slurry was treated with imidazole (sub stoichiometric) followed by the slow drop-wise addition of allyl bromide (4.85 g, 3.47 mL, 40.14 mmol, 1.5 eq). The resulting colourless solution was allowed to warm to RT and stir overnight. The reaction was shown to be complete by TLC analysis and subsequently quenched by the addition of water (15 mL). The volatile components were removed under vacuum and the residue formed was partitioned between ethyl acetate (50 mL) and water (50 mL). The layers were separated and the aqueous phase extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum to give a thick yellow oil. The oil was purified by silica gel column chromatography eluting with ethyl acetate and hexane (30:70). The product fractions were combined and the solvent was removed under vacuum to yield (±)-D-5-*O*-allyl-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-methylene **115** (12.7 g, 89% yield) as a thick colourless oil: R_f 0.45 (ethyl acetate and petroleum ether 40:60); δ_H (300 MHz, $CDCl_3$) 7.30-7.20 (10H, m, 2 × Ph), 7.17 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 6.79 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 5.80 (1H, ddt, *J* 17.1, 10.3, 5.6, $CH{=}CH_2$), 5.16 (1H, ddt, *J* 17.1, 1.8, 1.6, $CH{=}CH_xH_y$), 5.11 (1H, d, *J* 5.6, 3-H), 5.09 (1H, ddt, *J* 10.3, 1.8, 1.3, $CH{=}CH_xH_y$), 4.75 (1H, d, *J* 5.6, 3'-H), 4.58 (1H, d, J_{AB} 11.7, OCH_AH_B-Ph), 4.57 (2H, s, OCH_2-Ph), 4.61 (1H, d, J_{AB} 11.7, OCH_AH_B-Ph), 4.52 (1H, d, $J_{A'B'}$ 11.4, $OCH_{A'}H_{B'}$-$C_6H_4OCH_3$), 4.45 (1H, d, $J_{A'B'}$ 11.4, $OCH_{A'}H_{B'}$-$C_6H_4OCH_3$), 4.18-4.15 (2H, m, inositol ring), 4.05 (2H, ddd, *J* 5.6, 1.4, 1.4 $OCH_2CH{=}CH$), 3.76-3.73 (1H, m, inositol ring), 3.73 (3H, s, OCH_3), 3.45 (1H, dd, *J* 5.6, 5.6, inositol ring). The data are in good agreement with the literature values.[123]

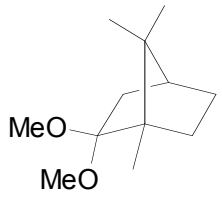
(±)-5-*O*-Allyl-2,6-*O*-dibenzyl-*myo*-inositol 116



116

(±)-D-5-*O*-Allyl-2,6-bis-*O*-benzyl-4-*O*-(4-methoxybenzyl)-*myo*-inositol 1,3-methylene **115** (2.86 g, 5.37 mmol, 1 eq) was dissolved in methanol (5 mL) and to this solution was added conc. hydrochloric acid (5 mL). The mixture was heated under reflux and stirred overnight. TLC analysis indicated the reaction had gone to completion, so the solution was cooled to 0 °C and the acid quenched by the cautious addition of solid sodium hydrogen carbonate (Note: copious effervescence is observed upon addition of sodium hydrogen carbonate) until pH 7 was achieved. The solid precipitate was removed by filtration and the filtrate concentrated under vacuum to give a crude colourless solid. This solid was purified by silica gel column chromatography eluting with ethyl acetate and hexane (30:70, then 40:60) to give (±)-5-*O*-allyl-2,6-*O*-dibenzyl-*myo*-inositol **116** (2.0 g 93% yield) as a colourless solid. R_f 0.45 (ethyl acetate); δ_H (300 MHz, $CDCl_3$) 7.33-7.18 (10H, m, 2 × Ph) 5.90 (1H, ddt, *J* 17.2, 10.4, 5.7, C*H*=CH_2), 5.23 (1H, ddt, J_{AB} 17.2, 1.7, 1.6, CH=CH_AH_B), 5.12 (1H, ddt, J_{AB} 10.4, 1.7, 1.3, CH=CH_AH_B), 4.85 (1H, d, J_{AB} 11.0, OC$H_A$$H_B$-Ph), 4.81 (1H, d, $J_{A'B'}$ 11.4, OC$H_{A'}$$H_{B'}$-Ph), 4.70 (1H, d, $J_{A'B'}$ 11.4, OC$H_{A'}$$H_{B'}$-Ph), 4.67 (1H, d, J_{AB} 11.0, OC$H_A$$H_B$-Ph), 4.28 (2H, dddd, *J* 21.4, 12.6, 5.5, 1.4, 1.4, OCH_2CH=CH_2), 3.95 (1H, t, *J* 2.8, 2-H), 3.73 (1H, t, *J* 9.5, inositol ring), 3.66 (1H, t, *J* 9.3, inositol ring), 3.50 (1H, dd, *J* 9.7, 2.7, inositol ring), 3.39 (1H, dd, *J* 9.7, 2.9, inositol ring), 3.15 (1H, dd, *J* 9.2, 9.2, inositol ring), 2.21 (3H, br s, OH). The data are in good agreement with the literature values.[123]

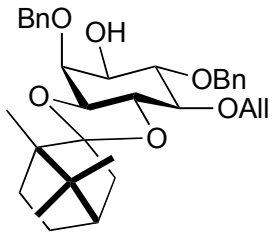
(1*S*)-(-)-Camphor dimethyl acetal 118



118

(1*S*)-(-)-Camphor **117** (10 g, 65.68 mmol, 1 eq) was dissolved in hexane (100 mL), to this solution was added Montmorillonite K-10 clay (18 g) followed by trimethyl orthoformate (27.88 g, 28.74 mL, 262.72 mmol, 4 eq). The resulting slurry was allowed to stir at RT overnight. The reaction was shown to be complete by TLC analysis and the mixture filtered through a pad of Celite®, washing with hexane (3 × 50 mL). The filtrate was concentrated under vacuum to yield (1*S*)-(-)-camphor dimethyl acetal **118** (12.3 g, 94% yield, 87% conversion to dimethyl acetal shown by ^{1}H NMR analysis) as a crude colourless oil: R_f 0.64 (diethyl ether and petroleum ether 30:70); δ_H (300 MHz, $CDCl_3$) 3.23 (3H, s, OCH_3), 3.17 (3H, s, OCH_3), 2.24-2.16 (1H, m, camphor ring), 1.80-1.63 (3H, m, camphor ring), 1.46-1.14 (3H, m, camphor ring), 0.97 (3H, s, 1-CH_3), 0.92 (3H, s, 7-CH_3), 0.83 (3H, s, 7-CH_3). The data are in good agreement with the literature values.[124]

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol (-)-104



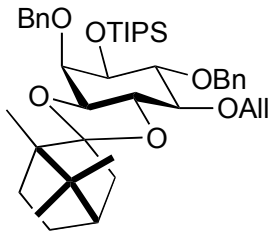
(-)-104

(±)-5-*O*-Allyl-2,6-*O*-dibenzyl-*myo*-inositol **116** (4.40 g, 11.00 mmol, 1 eq) was dissolved in dry CH_2Cl_2 (50 mL) under an atmosphere of nitrogen, to this was added (1*S*)-(-)-camphor dimethyl acetal **118** (7.52 g, 33.00 mmol, 3 eq, 87% converted to dimethyl acetal) and 4-toluenesulfonic acid monohydrate (105 mg, 0.55 mmol, 0.05 eq). The solution was heated under reflux and allowed to stir overnight. TLC analysis indicated the reaction had gone to completion, so the solution was allowed to cool to RT, the volatile components were removed under vacuum to give a colourless oil. The crude material was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (5:95, 6:94, 7:93, 8:92, 9:91, 10:90). The product fractions were combined and the solvent removed to yield (-)-1D-5-*O*-allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-104** (500 mg, 19% yield) as a colourless oil: R_f 0.37 (ethyl acetate/hexane 30:70); $[\alpha]_D^{20}$-10.5, (*c* 1.0 in $CHCl_3$) [(lit.[123] $[\alpha]_D^{22}$-11.7, (*c* 1.3 in $CHCl_3$)]; δ_H (300 MHz, $CDCl_3$) 7.43-7.29 (10H, m, 2 × Ph), 5.97 (1H, ddt, *J* 17.2, 10.4, 5.6, C*H*=CH_2), 5.33 (1H, ddt, *J* 17.2, 1.8, 1.7, CHC=C*H*H), 5.18 (1H, ddt, *J* 10.4, 1.7, 1.3, CHC=CH*H*), 5.03 (1H, d, J_{AB} 11.5, OC$H_A$$H_B$-Ph), 4.94 (1H, d, $J_{A'B'}$ 11.0, OC$H_{A'}$$H_{B'}$-Ph), 4.79 (1H, d, $J_{A'B'}$ 11.0, OC$H_{A'}$$H_{B'}$-Ph), 4.70 (1H, d, J_{AB} 11.5, OC$H_A$$H_B$-Ph), 4.41 (1H, ddt, J_{AB} 12.9, 5.6, 1.3, C$H_A$$H_B$=$CH_2$), 4.24-4.17 (2H, m, 1 × C$H_A$$H_B$=$CH_2$, 1 × inositol ring), 3.99 (1H, dd, *J* 9.6, 9.6, inositol ring), 3.73-3.49 (3 H, m, inositol ring), 3.31 (1H, dd, *J* 9.8,

1.8, inositol ring), 2.46 (1H, br s, OH), 2.17 (1H, dt, *J* 13.5, 3.3, camphor ring), 1.98-1.88 (1H, m, camphor ring), 1.79-1.69 (2H, m, 2 × camphor ring), 1.47 (1H, d, *J* 13.5, camphor ring), 1.30-1.23 (2H, m, 2 × camphor ring), 1.03 (3H, s, CH_3), 1.28-1.10 (3H, m, 3 × camphor ring), 0.86 (3H, s, CH_3 camphor bridge), 0.85 (3H, s, CH_3 camphor bridge). The data are in good agreement with the literature values.[123]

8.3. Towards the synthesis of 3-O-position compounds

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol (-)-121

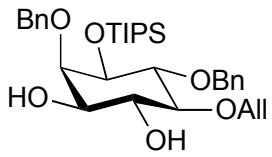


(-)-121

To a stirred solution of triisopropylsilane trifluoromethanesulfonate (2.26 g, 1.66 mL, 7.98 mmol, 4 eq) in dry CH_2Cl_2 (3 mL) under an atmosphere of nitrogen, was added triethylamine (944 mg, 1.3 mL, 9.35 mmol, 5 eq). The solution was allowed to stir for 1 h at RT, turning a dark orange colour, to this solution was added a solution of (-)-1D-5-*O*-allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-104** (3.0 g, 5.61 mmol, 1 eq) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred at RT overnight. TLC analysis indicated the reaction was complete, so it was diluted with CH_2Cl_2 (20 mL) and partitioned with the addition of water (50 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum to yield a dark brown oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography eluting with diethyl ether and petroleum ether (2:98) to give *(-)-1D-5-O-allyl-2,6-di-O-benzyl-1-O-triisopropylsilyl-3-O-exo-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol* **(-)-121** as a colourless oil (1.12 g, 87%). R_f 0.51 (ethyl acetate and hexane 10/90); $[\alpha]_D^{25}$ = -1.54 (*c* 1.23 in $CHCl_3$); ν_{max} (*NaCl disc*)/cm^{-1} 2943.5 (s), 2867.0 (s), 2362.9 (w), 1496.9 (w), 1454.2 (m), 1389.8 (m), 1369.4 (w), 1308.9 (w), 1245.9 (w), 1202.3 (w), 1181.76 (m), 1113.7 (s), 1068.2 (s),

921.6 (w), 883.4 (m), 820.3 (m), 730.4 (m), 695.5 (m), 680.7 (w); δ_H (300 MHz, $CDCl_3$) 7.38-7.12 (10H, m, Ar*H*), 5.81 (1H, dddd, *J* 17.2, 10.4, 5.5, 5.5, C*H*$_X$=CH$_Y$H$_Z$), 5.17 (1H, dd, *J* 17.2, 1.5, CH$_X$=C*H*$_Y$H$_Z$), 5.03 (1H, d, *J* 10.4, CH$_X$=CH$_Y$*H*$_Z$), 4.92 (1H, d, *J* 11.5, OC*H*$_A$H$_B$-Ph), 4.85 (1H, d, *J* 11.1, OC*H*$_{A'}$H$_{B'}$-Ph), 4.68 (1H, d, *J* 11.1, OCH$_{A'}$*H*$_{B'}$-Ph), 4.63 (1H, d, *J* 11.5, OCH$_A$*H*$_B$-Ph), 4.27 (1H, dd, *J* 12.7, 5.5, OC*H*$_V$*H*$_W$-CH=CH$_2$), 4.10 (1H, dd, *J* 2.7, 1.5, inositol ring, H-2) 4.03 (1H, dd, *J* 12.7, 5.5, OC*H*$_V$*H*$_W$-CH=CH$_2$), 3.94 (1H, dd, *J* 9.7, 9.7, inositol ring, H-6), 3.83 (1H, dd, *J* 9.0, 2.7, inositol ring, H-3), 3.67 (1H, dd, *J* 9.0, 9.0, inositol, H-4), 3.44 (1H, dd, *J* 9.7, 9.0, inositol ring, H-5), 3.21 (1H, dd, *J* 9.7, 1.5, inositol ring, H-5), 2.07 (1H, dt, *J* 13.4, 3.8, camphor ring), 1.89-1.77 (1H, m, camphor ring), 1.72-1.58 (2H, m, camphor ring), 1.43-1.36 (1H, m, camphor ring), 1.36-1.25 (1H, m, camphor ring), 1.18-1.09 (1H, m, camphor ring), 0.95-0.93 (21H, m, Si-(C*H*(C*H*$_3$)$_2$)$_3$), 0.93 (3H, s, C*H*-camphor ring), 0.77 (3H, s, C*H*$_3$-camphor bridge), 0.73 (3H, s, C*H*$_3$-camphor bridge); δ_C (75 MHz; $CDCl_3$), 139.9 (Ar*C*), 139.3 (Ar*C*), 135.8 (*C*H$_X$=CH$_Y$H$_Z$), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.7 (Ar*C*H), 127.4 (Ar*C*H), 120.7 (ketal carbon), 116.9 (CH$_X$=*C*H$_Y$H$_Z$), 83.7 (inositol ring), 81.9 (inositol ring), 77.9 (inositol ring), 77.2 (inositol ring), 76.4 (inositol ring), 76.3 (*C*H$_2$), 75.3 (inositol ring), 74.2 (*C*H$_2$), 72.1 (*C*H$_2$), 53.3 (*C*$_q$- camphor ring), 48.7 (*C*$_q$- camphor ring), 46.7 (*C*H$_2$), 45.3 (*C*H), 29.3 (*C*H$_2$), 27.1 (*C*H$_2$), 20.7 (*C*H$_3$), 20.5 (*C*H$_3$), 18.6 (*C*H$_3$), 13.3 (*C*H$_3$), 10.0 (*C*H); *m/z* (ES$^+$) [Found: (M+Na)$^+$ 713.4209. $C_{42}H_{62}O_6SiNa$ requires *M*$^+$, 713.4213], *m/z* (ES$^+$) 713 ([M+Na]$^+$, 100%); Anal. Calcd. For $C_{42}H_{62}O_6Si$: C, 73.0, H, 9.0; Found: C, 72.7, H, 9.3.

(-)-5-*O*-Allyl-2,6-bis-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol 105

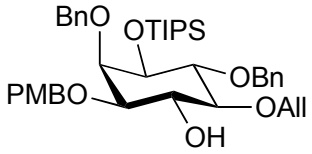


(-)-105

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-121** (5.17 g, 7.48 mmol, 1 eq) was dissolved in CH_2Cl_2 (90 mL) and methanol (60 mL) and stirred under an atmosphere of nitrogen. To this solution was added acetyl chloride (352 mg, 320 µL, 0.43 mmol, 0.6 eq) and the mixture was allowed to stir at room temperature for 6 h after which the reaction was shown to be complete by TLC analysis. The reaction was quenched with the addition of triethylamine (8 mL) and the volatile components removed under vacuum, resulting in a dark brown oil. The oil was partitioned between CH_2Cl_2 (100 mL) and water (100 mL), the layers separated and the aqueous phase further extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (magnesium sulfate), filtered and the filtrate concentrated under vacuum yielding a brown oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (20:80). The product fractions were combined and the solvent removed under vacuum to give *(-)-5-O-allyl-2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol* **(-)-105** (3.8 g, 91%) as a colourless oil: R_f 0.51 (ethyl acetate/petroleum ether 50/50); $[\alpha]_D^{25}$= -11.9, (*c* 1.2 in $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 7.42-7.27 (10H, m, Ar*H*), 5.87 (1H, dddd, *J* 17.2, 10.5, 5.7, 5.7, CH_X=CH_YH$_Z$), 5.19 (1H, dddd, *J* 17.2, 1.6, 1.4, 1.4, CH$_X$=CH_YH$_Z$), 5.11 (1H, dddd, *J* 10.5, 1.6, 1.4, 1.4, CH$_X$=CH$_Y$$H_Z$), 5.10 (1H, d, *J* 11.2, OCH_AH$_B$-Ph), 4.90 (1H, d, *J* 11.7, OC$H_{A'}$H$_{B'}$-Ph), 4.84 (1H, d, *J* 11.7, OCH$_{A'}$$H_{B'}$-Ph), 4.73 (1H, d, *J* 11.2, OCH$_A$$H_B$-Ph), 4.31 (1H, dddd, *J* 12.3, 5.7, 1.4, 1.4, OCH_VH$_W$-CH=CH$_2$), 4.24 (1H, dddd, *J* 12.3, 5.7, 1.4, 1.4, OCH$_V$$H_W$-CH=CH$_2$), 4.00 (1H, dd, *J* 2.6, 1.2, inositol ring, H-2), 3.88-3.82 (2H, m, inositol ring, H-4 and 5), 3.81 (1H, dd, *J* 9.5, 9.5,

inositol ring, H-6), 3.47 (1H, dd, *J* 9.5, 2.6, inositol ring, H-1), 3.24-3.15 (1H, m, inositol, H-3), 2.83 (1H, br s, OH), 2.57 (1H, br s, OH), 1.02-0.93 (21H, m, Si-[C*H*($C*H*_3$)$_2$]$_3$); δ_C (75 MHz; $CDCl_3$), 139.1 (Ar*C*), 138.9 (Ar*C*), 135.0 (*C*H_X=$CH_Y$$H_Z$), 128.4 (Ar*C*H), 128.0 (Ar*C*H), 127.6 (Ar*C*H), 127.4 (Ar*C*H), 127.1 (Ar*C*H), 117.0 (CH_X=*C*$H_Y$$H_Z$), 83.4 (inositol ring), 81.7 (inositol ring), 81.5 (inositol ring), 75.3 (O*C*H_2-Ph), 75.2 (O*C*H_2-Ph), 74.5 (inositol ring), 74.3 (inositol ring), 74.3 (*C*H_2CH=CH_2), 72.3 (inositol ring), 18.3 (TIPS-*C*H_3), 18.2 (TIPS-*C*H_3), 12.9 (TIPS-*C*H); *m/z* (ES^+) [Found: $(M+Na)^+$ 579.3116. $C_{32}H_{48}O_6SiNa$ requires M^+, 579.3118], *m/z* (ES^+) 579 ($[M+Na]^+$, 100%); Anal. Calcd. For $C_{32}H_{48}O_6Si$: C, 69.0, H, 8.7; Found: C, 69.1, H, 8.8. The data are in good agreement with the literature values.[31]

(-)-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-1-*O*-triisopropylsilyl *myo*-inositol (-)-122



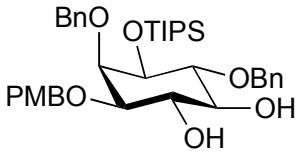
(-)-122

Dibutyltin oxide (246 mg, 0.99 mmol, 1.1 eq) and cesium fluoride (273 mg, 1.80 mmol, 2 eq) were added to a solution of (-)-5-*O*-allyl-2,6-bis-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol **(-)-105** (555 mg, 0.90 mmol, 1 eq) in dry toluene (20 mL). Soxhlet apparatus containing 3 Å molecular sieves was attached and the mixture was heated under reflux overnight to pre-form the tin acetal complex. The mixture was cooled to room temperature and the volatile components removed under vacuum. The resulting waxy yellow solid was dissolved in dry DMF and 4-(methoxy)benzyl chloride (155 mg, 134 µL, 0.99 mmol, 1.1 eq) added. The reaction mixture was allowed to stir at room temperature and monitored by TLC analysis. After 48 h, 4-(methoxy)benzyl chloride (141 mg, 122

μL, 0.90 mmol, 1 eq) was added and the reaction heated to 50 °C for 12 h before the addition of (tetrabutyl)ammonium iodide (332 mg, 0.90 mmol, 1 eq). After a further 6 h of stirring at 50 °C the reaction was adjudged to be complete by TLC analysis. The volatile components were removed under vacuum and the residue partitioned between ethyl acetate (50 mL) and water (50 mL). The partitioning produced a fine precipitate preventing separation of the layers. The emulsion was clarified by filtration through Celite and consequently easy separation of the layers was achieved. The aqueous phase was further extracted with ethyl acetate (3 × 20 mL). The combined organic components were washed with brine and dried (magnesium sulfate), filtered and the solvent removed under vacuum. The crude brown oil was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (20:80). The mixed fractions combined and re-columned eluting with ethyl acetate and petroleum ether (15:85 then 20:80), this gave the major regioisomer *(-)-5-O-allyl-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-1-O-triisopropylsilyl myo-inositol* **(-)-122** (267 mg, 43%) as a colourless solid: R_f 0.58 (ethyl acetate/petroleum ether 30/70); $[\alpha]_D^{25}$ = -6.3, (*c* 1.5 in $CHCl_3$); υ_{max} (*NaCl disc*)/cm^{-1} 3556 (m), 3453 (m broad), 2943 (s), 2866 (s), 1948 (w), 1879 (w), 1646 (m), 1514 (s), 1211 (s), 1015 (s); δ_H (300 MHz, $CDCl_3$) 7.32 (2H, d, *J* 8.7, $OCH_2C_6\mathit{H}_4OCH_3$), 7.29-7.12 (10H, m, Ar*H*), 6.70 (2H, d, *J* 8.7, $OCH_2C_6\mathit{H}_4OCH_3$), 5.80 (1H, dddd, *J* 17.2, 10.6, 5.5, 5.5, $C\mathit{H}_X{=}CH_YH_Z$), 5.11 (1H, dddd, *J* 17.2, 1.6, 1.6, 1.6, $CH_X{=}C\mathit{H}_YH_Z$), 5.00 (1H, dddd, *J* 10.6, 1.6, 1.6, 1.6, $CH_X{=}CH_Y\mathit{H}_Z$), 4.79 (1H, d, *J* 11.6, $OC\mathit{H}_AH_B$-Ph), 4.78 (1H, d, *J* 11.4, $OC\mathit{H}_{A'}H_{B'}$-Ph), 4.73 (1H, d, *J* 11.6, $OCH_A\mathit{H}_B$-Ph), 4.73 (1H, d, *J* 11.4, $OCH_{A'}\mathit{H}_{B'}$-Ph), 4.53 (2H, s, $OC\mathit{H}_2$-$C_6H_4OCH_3$), 4.23 (1H, dddd, *J* 12.4, 5.5, 1.6, 1.6, $OC\mathit{H}_VH_W$-$CH{=}CH_2$), 4.17 (1H, dddd, *J* 12.4, 5.5, 1.6, 1.6, $OCH_V\mathit{H}_W$-$CH{=}CH_2$), 4.00 (1H, dd, *J* 9.4, 9.4, inositol ring, H-4), 3.80 (1H, dd, *J* 2.1, 2.1, inositol ring, H-2), 3.78 (1H, dd, *J* 9.4, 9.4, inositol ring, H-6), 3.73 (3H, s, OCH_2-$C_6H_4OC\mathit{H}_3$), 3.62 (1H, dd, *J* 9.4, 2.1, inositol ring, H-1), 3.14 (1H, dd, *J* 9.4, 9.4, inositol ring, H-5), 3.13 (1H, dd, *J* 9.4, 2.1, inositol ring, H-3), 2.41 (1H, br s, 4-O*H*), 0.99-0.93 (21H, m, Si-[C*H*(CH_3)$_2$]$_3$); δ_C (75 MHz; $CDCl_3$), 159.8 (Ar*C*), 139.7 (Ar*C*), 135.7 ($CH_A{=}CH_BH_C$), 130.5 (Ar*C*), 130.0 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 127.7

(Ar*C*H), 127.6 (Ar*C*H), 127.4 (Ar*C*H), 117.1 (CH=*C*H_2), 114.4 (PMB – Ar*C*H), 84.2 (inositol ring), 82.1 (inositol ring), 80.2 (inositol ring), 79.4 (inositol ring), 75.7 (O*C*H_2-Ph), 74.9 (O*C*H_2CH=CH_2), 74.6 (O*C*H_2-Ph), 74.5 (inositol ring), 73.5 (inositol ring), 72.6 (O*C*H_2-$C_6H_4OCH_3$), 55.7 (OCH_2-C_6H_4O*C*H_3), 18.6 (TIPS-*C*H_3), 18.6 (TIPS-*C*H_3), 13.2 (TIPS-*C*H); *m/z* (ES^+) [Found: $(M+Na)^+$ 699.3680. $C_{40}H_{56}O_7SiNa$ requires M^+, 699.3693], *m/z* (ES^+) 699 ($[M+Na]^+$, 100%); Anal. Calcd. For $C_{40}H_{56}O_7Si$: C, 71.0, H, 8.3; Found: C, 70.9, H, 8.4.

(-)-2,6-Bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-1-*O*-triisopropylsilyl *myo*-inositol (-)-128

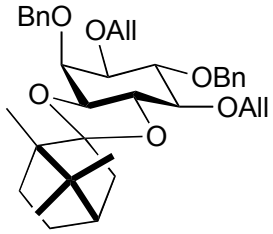


(-)-128

(-)-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-1-*O*-triisopropylsilyl *myo*-inositol **(-)-122** (100 mg, 0.15 mmol, 1 eq) was dissolved in absolute ethanol (2 mL), to this (under an atmosphere of nitrogen) was added diisopropylethyl amine (19 mg, 26 µL, 0.15 mmol, 1 eq). The solution was stirred at room temperature for 5 minutes and then Wilkinson's catalyst (27 mg, 0.03 mmol, 0.2 eq) was added. The dark red slurry was heated under reflux for 3 h before being cooled to room temperature and filtered though Celite. The filtrate was concentrated under vacuum and the residue analysed by ^1H NMR to confirm complete isomerisation of the allylic ether to the enol ether. The crude residue was dissolved in MeOH (2 mL) and 4-toluenesulfonic acid monohydrate (9 mg, 0.05 mmol, 0.3 eq) was added. The reaction was left to stir at room temperature for 4 h before the addition of triethylamine (0.5 mL) to quench the acid. The volatile components were removed under vacuum and the residue purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (30:70), to give

(-)-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-1-O-triisopropylsilyl myo-inositol **(-)-128** (72 mg, 76%) as a pale yellow oil. δ_H (300 MHz, $CDCl_3$) 7.33 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 7.30-7.17 (10H, m, 2 × Ph), 6.80 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 4.84 (1H, d, *J* 11.8, OCH_AH_B-Ph), 4.79 (1H, d, *J* 11.5, $OCH_{A'}H_{B'}$-Ph), 4.74 (1H, d, *J* 11.5, $OCH_{A'}H_{B'}$-Ph), 4.66 (1H, d, *J* 11.8, OCH_AH_B-Ph), 4.53 (2H, s, OCH_AH_B-$C_6H_4OCH_3$), 3.91 (1H, dd, *J* 9.4, 9.4, inositol ring, H-4), 3.83 (1H, dd, *J* 2.1, 2.1, inositol ring, H-2), 3.77-3.70 (4H, m, OCH_2-$C_6H_4OCH_3$ + inositol ring, H-6), 3.66 (1H, dd, *J* 9.5, 2.1, inositol ring, H-1), 3.31 (1H, dd, *J* 9.3, 9.3, inositol ring, H-5), 3.15 (1H, dd, *J* 9.3, 2.1, inositol ring, H-3), 2.31 (2H, br s, 2 × OH), 1.04-0.98 (21H, m, Si-[$CH(CH_3)_2]_3$). Compound was not pure and could not be synthesised reliably for full characterisation.

(-)-1D-1,5-bis-*O*-Allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol (-)-130

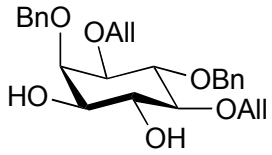


(-)-130

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-104** (1.19 g, 2.22 mmol, 1 eq) was dissolved in dry THF (25 mL), the solution was cooled to 0 °C and sodium hydride (60% dispersion in mineral oil, 106 mg, 2.67 mmol, 1.2 eq) added. The resulting suspension was warmed to room temperature and stirred for 1 h, then re-cooled to 0 °C. Imidazole (substoichiometric), tetrabutylammonium iodide (substoichiometric) and allyl bromide (323 mg, 231 µL, 2.67 mmol, 1.2 eq) were added to the cold suspension and the reaction allowed to warm to room temperature with stirring. After 1 h, dry DMF (10 mL) was added and the reaction left overnight. TLC

analysis indicated the reaction had gone to completion, so the volatile components were removed under reduced pressure. The residue was partitioned between ethyl acetate (50 mL) and water (50 mL), the layers separated and the aqueous phase was further extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with brine, dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (5/95). This afforded (-)-1D-1,5-bis-*O*-allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-130** (781 mg, 61% yield) as a colourless oil: R_f 0.46 (ethyl acetate/petroleum ether 20:80); $[\alpha]_D^{25}$ = -23.3, (*c* 1.4 in $CHCl_3$) [(Lit.[91] $[\alpha]_D^{26}$ = -23.0, (*c* 0.49 in $CHCl_3$)]; δ_H (300 MHz, $CDCl_3$) 7.49-7.26 (10H, m, Ar*H*), 6.06-5.84 (2H, m, CH_X=CH_YH$_Z$, C$H_{X'}$=C$H_{Y'}$H$_{Z'}$), 5.35 (1H, dddd, *J* 16.8, 1.5, 1.5, 1.5, CH_X=CH_YH$_Z$), 5.29 (1H, dddd, *J* 16.7, 1.5, 1.5, 1.5, C$H_{X'}$=C$H_{Y'}$H$_{Z'}$), 5.18 (2H, dd, *J* 10.4, 1.5, CH_X=CH_Y*H*$_Z$ + C$H_{X'}$=C$H_{Y'}$*H*$_{Z'}$), 4.93 (1H, d, *J* 12.3, OC*H*$_A$H$_B$-Ph), 4.87 (1H, d, *J* 10.5, OC*H*$_{A'}$H$_{B'}$-Ph), 4.86 (1H, d, *J* 12.3, OCH$_A$*H*$_B$-Ph), 4.83 (1H, d, *J* 10.5, OCH$_{A'}$*H*$_{B'}$-Ph), 4.40 (1H, dddd, *J* 13.1, 5.3, 1.5, 1.5, OC*H*$_V$H$_W$-CH=CH$_2$), 4.27-4.18 (2H, OCH$_V$*H*$_W$-CH=CH$_2$ + OC*H*$_{V'}$H$_{W'}$-CH=CH$_2$), 4.09-4.00 (3H, m, OCH$_{V'}$*H*$_{W'}$-CH=CH$_2$ + 2 × inositol ring, H-2 and 4), 3.84 (1H, dd, *J* 9.3, 8.6, inositol ring, H-5), 3.50 (1H, dd, *J* 9.5, 8.6, inositol ring, H-6), 3.42 (1H, dd, *J* 9.5, 3.2, inositol ring, H-1), 3.22 (1H, dd, *J* 9.8, 1.7, inositol ring, H-3), 2.15 (1H, dt, *J* 13.4, 3.5, camphor ring), 2.01-1.91 (1H, m, camphor ring), 1.80-1.68 (2H, m, camphor ring), 1.49-1.37 (2H, m, camphor ring), 1.29-1.19 (1H, m, camphor ring), 1.04 (3H, s, C*H*$_3$ camphor ring), 0.90 (3H, s, C*H*$_3$-camphor bridge), 0.88 (3H, s, C*H*$_3$ camphor bridge). The data are in good agreement with the literature values.[91]

(-)-1D-1,5-bis-*O*-Allyl-2,6-bis-*O*-benzyl-*myo*-inositol (-)-129



(-)-129

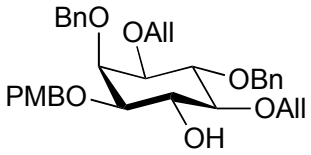
Method 1

(-)-1D-1,5-bis-*O*-Allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo-[2.2.1]-hept-2'-ylidine)-*myo*-inositol **(-)-130** (781 mg, 1.36 mmol, 1 eq) was dissolved in CH_2Cl_2 (15 mL) and methanol (10 mL). To this solution was added acetyl chloride (64 mg, 58 µL, 0.82 mmol, 0.6 eq). The reaction was stirred at room temperature for 6 h before being quenched with the addition of triethylamine (0.5 mL). The volatile components were removed under reduced pressure and the resulting residue purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (30:70). This afforded (-)-1D-1,5-bis-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **(-)-129** (560 mg, 93% yield) as a colourless solid: R_f 0.56 (ethyl acetate); δ_H (300 MHz, $CDCl_3$) 7.42-7.29 (10H, m, Ar*H*), 6.03-5.88 (2H, m, CH_X=CH$_Y$H$_Z$, C$H_{X'}$=CH$_{Y'}$H$_{Z'}$), 5.34 (1H, dddd, *J* 17.2, 1.5, 1.5, 1.5, CH$_X$=CH_YH$_Z$), 5.29 (1H, dddd, *J* 17.2, 1.5, 1.5, 1.5, CH$_{X'}$=C$H_{Y'}$H$_{Z'}$), 5.20 (1H, dddd, *J* 10.3, 1.4, 1.4, 1.4, CH$_X$=CH$_Y$$H_Z$), 5.18 (1H, dddd, *J* 10.2, 1.5, 1.4, 1.4, CH$_{X'}$=CH$_{Y'}$$H_{Z'}$), 5.06 (1H, d, *J* 11.5, OCH_AH$_B$-Ph), 4.90 (1H, d, *J* 12.5, OC$H_{A'}$H$_{B'}$-Ph), 4.79 (1H, d, *J* 12.5, OCH$_{A'}$$H_{B'}$-Ph), 4.68 (1H, d, *J* 11.5, OCH$_A$$H_B$-Ph), 4.41 (1H, dddd, *J* 12.5, 5.7, 1.3, 1.3, OCH_VH$_W$-CH=CH$_2$), 4.27 (1H, dddd, *J* 12.5, 5.7, 1.3, 1.3, OCH$_V$$H_W$-CH=CH$_2$) 4.03-4.17 (2H, m, OC$H_{V'}$$H_{W'}$-CH=CH$_2$), 4.03 (1H, dd, *J* 2.5, 2.5, inositol ring, H-2), 3.92 (1H, dd, *J* 9.6, 9.6, inositol ring, H-6), 3.79 (1H, ddd, *J* 9.6, 9.6, 1.7, inositol ring, H-4), 3.40 (1H, ddd, *J* 9.6, 8.1, 2.5, inositol ring, H-3), 3.37 (1H, dd, *J* 9.6, 2.5, inositol ring, H-1), 3.19 (1H, dd, *J* 9.6, 9.6, inositol ring, H-5), 2.53 (1H, d, *J* 1.7, 4-O*H*), 2.28 (1H, d, *J* 8.1, 3-O*H*). The data are in good agreement with the literature values.[91]

Method 2

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-130** (150 mg, 281 μmol, 1 eq) was dissolved in dry DMF (5 mL), the solution was cooled to 0 °C and sodium hydride (60% dispersion in mineral oil, 13 mg, 561 μmol, 2 eq), allyl bromide (67 mg, 49 μL, 561 μmol, 2 eq) and tetrabutylammonium iodide (substoichiometric) were added. The reaction allowed to warm to room temperature and allowed to stir overnight. TLC analysis indicated the reaction had gone to completion, methanol was added to quench the sodium hydride. The volatile components were removed under reduced pressure and the residue partitioned between CH_2Cl_2 (5 mL) and water (5 mL). The layers separated and the aqueous phase was further extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers dried (magnesium sulfate), filtered and the filtrate was concentrated under vacuum. The resulting residue was dissolved in CH_2Cl_2 (3 mL) and methanol (2 mL) and to this solution was added acetyl chloride (13 mg, 12 μL, 168 μmol, 0.6 eq). The reaction was stirred at room temperature for 6 h before being quenched with the addition of triethylamine (0.5 mL). The volatile components were removed under reduced pressure and the resulting residue purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (30:70). This afforded (-)-1D-1,5-bis-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **(-)-129** (115 mg, 93% yield) as a colourless solid. The analysis of this material was consistent with that found for the method 1.

(+)-1D-1,5-bis-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol (+)-131

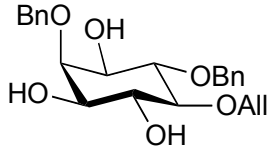


(+)-131

(-)-1D-1,5-bis-O-Allyl-2,6-bis-O-benzyl-*myo*-inositol (-)-**129** (100mg, 0.227mmol, 1 eq) was dissolved in dry acetonitrile (30 mL). To this solution was added dibutyltin oxide (85 mg, 0.34 mmol, 1.5 eq) and TBABr (73 mg, 0.227 mmol, 1 eq) and a Soxhlet apparatus containing 3 Å molecular sieves attached. The mixture was heated under reflux for 2 h before being cooled to room temperature and 4-(methoxy)benzyl chloride (170 mg, 148 µL, 1.1 mmol, 4.8 eq) added. The reaction was then heated under reflux overnight. The reaction was adjudged complete by TLC analysis so the volatile components were removed under reduced pressure. The residue was then partitioned between ethyl acetate (50 mL) and water (50 mL), the layers separated and the aqueous layer further extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with a saturated aqueous solution of sodium hydrogen carbonate (50 mL) and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (10:90 then 20:80) to give *(+)-1D-1,5-bis-O-allyl-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-myo-inositol* ***(-)-129*** (32 mg, 25% yield) as a colourless gum: R_f 0.46 (petroleum ether / ethyl acetate 70/30); $[\alpha]_D^{25}$= +3.6, (*c* 0.9 in $CHCl_3$); υ_{max} (*KBr disc*)/cm^{-1} 3548 (s), 3361 (s), 3083 (w), 3062 (w), 2913 (s), 2896 (s), 2868 (s), 1951 (w), 1887 (w), 1818, (w), 1616 (m), 1516 (s), 1072 (s); δ_H (300 MHz, $CDCl_3$) 7.46-7.23 (12H, m, Ar*H*), 6.92-6.86 (2H, m, $OCH_2C_6H_4OCH_3$), 5.97 (2H, dddd, *J* 17.2, 10.9, 5.6, 5.6, CH_X=CH_YH_Z and $CH_{X'}$=$CH_{Y'}H_{Z'}$), 5.33 (1H, dddd, *J* 17.2, 1.7, 1.7, 1.7, CH_X=CH_YH_Z), 5.30 (1H, dddd, *J* 17.2, 1.7, 1.7, 1.7, $CH_{X'}$=$CH_{Y'}H_{Z'}$), 5.20 (1H,

dddd, *J* 10.9, 1.7, 1.4, 1.4, CH_A=$CH_B$$H_C$), 5.18 (1H, dddd, *J* 10.4, 1.6, 1.3, 1.3, $CH_{A'}$=$CH_{B'}$$H_{C'}$), 4.90 (1H, d, *J* 12.0, OC$H_A$$H_B$-Ph), 4.89 (1H, d, *J* 10.5, OC$H_{A'}$$H_{B'}$-Ph), 4.80 (1H, d, *J* 12.0, OC$H_A$$H_B$-Ph), 4.80 (1H, d, *J* 10.5, OC$H_{A'}$$H_{B'}$-Ph), 4.56 (1H, d, *J* 11.4, OC$H_{A''}$$H_{B''}$-$C_6H_4OCH_3$), 4.49 (1H, d, *J* 11.4, OC$H_{A''}$$H_{B''}$-$C_6H_4OCH_3$), 4.42-4.31 (2H, m, OC$H_X$$H_Y$-CH=$CH_2$), 4.18-4.07 (3H, m, OC$H_{X'}$$H_{Y'}$-CH=$CH_2$ + 1 × inositol ring, H-4), 4.03 (1H, dd, *J* 2.3, 2.3, inositol ring, H-2), 3.96 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.82 (3H, s, OCH_2-$C_6H_4OCH_3$), 3.26 (1H, dd, *J* 9.5, 2.3, inositol ring, H-1), 3.24 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5), 3.18 (1H, dd, *J* 9.9, 2.3, inositol ring, H-3), 2.57 (1H, br s, O*H*); δ_C (100 MHz; $CDCl_3$) 159.3 (PMB – Ar*C*), 138.9 (Ar*C*) 138.9 (Ar*C*), 135.4 (*C*H_X=$CH_Y$$H_Z$), 134.9 (*C*$H_{X'}$=$CH_{Y'}$$H_{Z'}$) 130.0 (Ar*C*H), 129.4 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 127.8 (Ar*C*H), 127.6 (Ar*C*H), 127.4 (Ar*C*H), 116.7 (CH_X=*C*$H_Y$$H_Z$ + $CH_{X'}$=*C*$H_{Y'}$$H_{Z'}$), 113.9 (PMB – Ar*C*H), 82.9 (inositol ring, C-5), 81.4 (inositol ring, C-6), 80.8 (inositol ring, C-1), 79.8 (inositol ring, C-3), 75.8 (O*C*H_2-Ph), 74.2 (*C*H_2-C=CH_2), 74.0 (O*C*H_2-Ph), 73.5 (inositol ring, C-2), 72.6 (inositol ring, C-4), 72.0 (O*C*H_2-$C_6H_4OCH_3$), 71.8 (*C*H_2-C=CH_2), 55.3 (OCH_2-C_6H_4O*C*H_3); *m/z* (ES^+) [Found: $(M+Na)^+$ 583.2665. $C_{34}H_{40}O_7Na$ requires M^+, 583.2666], *m/z* (ES^+) 662 ($[M+HNEt_3]^+$, 100%), 583 ($[M+Na]^+$, 60%), 578 ($[M+NH_4]^+$, 45%); Anal. Calcd. For $C_{34}H_{40}O_7$: C, 72.8, H, 7.2; Found: C, 72.9, H, 7.3.

(-)-5-*O*-Allyl-2,6-*O*-dibenzyl-*myo*-inositol 138

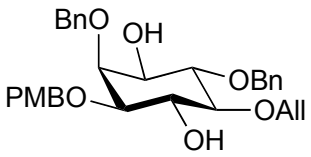


(-)-138

(-)-1D-5-*O*-allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-104** (2.86 g, 5.37 mmol, 1 eq) was dissolved in methanol (5 mL) and to this solution was added conc. hydrochloric acid (5 mL). The

solution was heated under reflux and stirred overnight. TLC analysis indicated the reaction had gone to completion, so the solution was cooled to 0 °C and the acid quenched by the cautious addition of solid sodium hydrogen carbonate (Note: copious effervescence is observed upon addition of sodium hydrogen carbonate) until pH 7 was achieved. The solid precipitate was removed by filtration and the filtrate concentrated under vacuum to give a crude colourless solid. This solid was purified by silica gel column chromatography eluting with ethyl acetate and hexane (30:70, then 40:60) to give (-)-5-*O*-allyl-2,6-*O*-dibenzyl-*myo*-inositol **138** (2.0 g 93% yield) as a colourless solid: R_f 0.45 (ethyl acetate); $[\alpha]_D^{20}$ = -17.6 (*c* 1.00 in $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.42-7.28 (10H, m, Ar*H*), 5.98 (1H, dddd, *J* 17.2, 10.4, 5.7, 5.7, CH_X=CH_YH$_Z$), 5.31 (1H, dddd, *J* 17.2, 1.4, 1.4, 1.4, CH$_X$=CH_YH$_Z$), 5.20 (1H, dddd, *J* 10.4, 1.4, 1.4, 1.4, CH$_X$=CH$_Y$$H_Z$), 4.93 (1H, d, *J* 11.2, OCH_AH$_B$-Ph), 4.89 (1H, d, *J* 11.6, OC$H_{A'}$H$_{B'}$-Ph), 4.78 (1H, d, *J* 11.2, OCH$_A$$H_B$-Ph), 4.76 (1H, d, *J* 11.6, OCH$_{A'}$$H_{B'}$-Ph), 4.39 (1H, dddd, *J* 12.5, 5.7, 1.4, 1.4, OCH_2CH=CH$_2$), 4.32 (1H, dddd, *J* 12.5, 5.7, 1.4, 1.4, OCH_2CH=CH$_2$), 4.03 (1H, dd, *J* 2.8, 2.8, inositol ring, H-2), 3.81 (1H, ddd, *J* 9.5, 9.5, 2.3, inositol ring, H-4), 3.74 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.58 (1H, ddd, *J* 9.5, 5.0, 2.8, inositol ring, H-1), 3.48 (1H, ddd, *J* 9.5, 7.1, 2.8, inositol ring, H-3), 3.23 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5), 2.57 (1H, d, *J* 2.3, 4-O*H*), 2.36 (1H, d, *J* 7.1, 3-O*H*), 2.33 (1H, d, *J* 5.0, 1-O*H*). The data are in good agreement with the literature values.[89]

(-)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol (-)-139

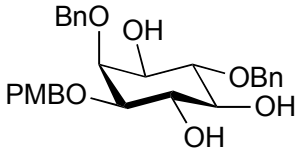


(-)-139

Dibutyltin oxide (465 mg, 1.87 mmol, 1.5 eq) was added to a solution of (-)-1D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **(-)-138** (500 mg, 1.25 mmol, 1 eq) in dry toluene (30 mL). A Soxhlet apparatus containing 3 Å molecular sieves was attached and the mixture was heated under reflux overnight to pre-form the tin acetal complex. The mixture was cooled to room temperature and the volatile components removed under vacuum. The resulting residue was dissolved in dry DMF (30 mL) and TBAI (462 mg, 1.25 mmol, 1 eq), cesium fluoride (380 mg, 2.50 mmol, 2 eq) and 4-(methoxy)benzyl chloride (391 mg, 339 µL, 2.50 mmol, 2 eq) were added. The reaction was heated to 50 °C and left to stir overnight. The reaction was adjudged to be complete by TLC analysis and cooled to RT. The volatile components were removed under vacuum and the residue partitioned between ethyl acetate (50 mL) and water (50 mL), the layers were separated and the aqueous phase extracted with ethyl acetate (3 × 30 mL). The combined organic components were dried (magnesium sulfate), filtered and the solvent removed under vacuum. The crude material was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (20:80 then 30:70). This gave *(-)-1D-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-myo-inositol* **(-)-139** (325 mg, 50% yield) as a colourless solid: R_f 0.48 (petroleum ether / ethyl acetate 60/40); mp 98-100 ºC (recrystallised from ethyl acetate), $[\alpha]_D^{25}$ = -11.8 (*c* 0.70 in $CHCl_3$); υ_{max} (*KBr disc*)/cm^{-1} 3454 (s, broad), 3063 (w), 3030 (w), 2962 (s), 2921 (s), 2853 (m), 1953 (w), 1722 (m), 1612 (s), 1514 (s), 1259 (s), 1066 (s); δ_H (300 MHz, $CDCl_3$) 7.42-7.26 (12H, m, Ar*H*), 6.91 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 6.01 (1H, dddd, *J* 17.2, 10.4, 5.7, 5.7, CH_X=CH_YH_Z), 5.32 (1H, dddd, *J* 17.2, 1.6, 1.4, 1.4, CH_X=CH_YH_Z), 5.20 (1H, dddd, *J* 10.4, 1.6, 1.4,

1.4, CH_X=$CH_Y$$H_Z$), 4.94 (1H, d, *J* 11.5, OC$H_A$$H_B$-Ph), 4.93 (1H, d, *J* 11.1, OC$H_{A'}$$H_{B'}$-Ph), 4.77 (1H, d, *J* 11.1, OC$H_{A'}$$H_{B'}$-Ph), 4.72 (1H, d, *J* 11.5, OC$H_A$$H_B$-Ph), 4.65 (1H, d, *J* 11.4, OC$H_A$$H_B$-$C_6H_4OCH_3$), 4.55 (1H, d, *J* 11.4, OC$H_A$$H_B$-$C_6H_4OCH_3$), 4.40 (1H, dddd, *J* 12.5, 5.7, 1.4, 1.4, OC$H_V$$H_W$-CH=$CH_2$), 4.35 (1H, dddd, *J* 12.5, 5.7, 1.4, 1.4, OC$H_V$$H_W$-CH=$CH_2$), 4.09 (1H, ddd, *J* 9.5, 9.5, 1.9, inositol ring, H-4), 4.06 (1H, dd, *J* 2.6, 2.6, inositol ring, H-2), 3.84 (3H, s, OCH_2-$C_6H_4OCH_3$), 3.75 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.51 (1H, ddd, *J* 9.5, 6.3, 2.6, inositol ring, H-1), 3.28 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5), 3.27 (1H, dd, *J* 9.5, 2.6, inositol ring, H-3), 2.54 (1H, d, *J* 1.9, 4-O*H*), 2.29 (1H, d, *J* 6.3, 1-O*H*). δ_C (75 MHz; $CDCl_3$), 138.6 (Ar*C*), 138.6 (Ar*C*), 135.2 (CH_2*C*H=CH_2), 129.8 (Ar*C*), 129.5 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.1 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.7 (Ar*C*H), 116.9 (CH_2*C*H=CH_2), 114.0 (Ar*C*H), 82.9 (inositol ring, C-5), 81.9 (inositol ring, C-6), 80.0 (inositol ring, C-3), 76.1 (inositol ring, C-2), 75.5 (O*C*H_2-Ph), 74.6 (O*C*H_2-Ph), 74.1 (*C*H_2CH=CH_2), 73.0 (inositol ring, C-4), 72.6 (inositol ring, C-1), 72.2 (O*C*H_2-$C_6H_4OCH_3$), 55.3 (OCH_2-C_6H_4O*C*H_3); *m/z* (ES^+) [Found: $(M+Na)^+$ 543.2358. $C_{31}H_{36}O_7Na$ requires M^+, 543.2359], *m/z* (ES^+) 543 ($[M+Na]^+$, 100%); Anal. Calcd. For $C_{31}H_{36}O_7$: C, 71.5, H, 7.0; Found: C, 71.7, H, 6.9.

(+)-1D-2,6-bis-*O*-Benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol (+)-106



(+)-106

Method 1

(-)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol **(-)-139** (300 mg, 0.58 mmol, 1 eq) was dissolved in absolute ethanol (20 mL) under a blanket of nitrogen, to this solution was added diisopropylethyl amine (75 mg, 101 µL, 0.58 mmol, 1 eq). The solution was stirred at room temperature for 5

minutes and then Wilkinson's catalyst (111 mg, 0.12 mmol, 0.2 eq) was added. The dark red slurry was heated under reflux for 3 h before being cooled to room temperature and filtered though Celite®. The filtrate was concentrated under vacuum and the residue analysed by 1H NMR to confirm complete isomerisation of the allylic ether to the enol ether. The crude residue was dissolved in methanol/CH_2Cl_2 (3:2, 20 mL) and acetyl chloride (27 mg, 24 µL, 0.35 mmol, 0.6 eq) was added. The reaction was left to stir at room temperature for 4 h before the addition of triethylamine (2 mL) to quench the acid. The mixture was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (50:50 then 60:40) to afford a pale yellow solid. Recrystallisation from ethyl acetate and hexane gave *(+)-1D-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-myo-inositol* ***(+)-106*** (100 mg, 36% yield) as a colourless crystalline solid: R_f 0.56 (ethyl acetate); mp 126-128 ºC (recrystallised from ethyl acetate); $[\alpha]_D^{20}$ = +10.5 (*c* 0.86 in $CHCl_3$); υ_{max} (*KBr disc*)/cm^{-1} 3395 (s, broad), 3084 (w), 3024 (m), 2943 (m), 2885 (m), 1514 (s), 1249 (s), 1064 (s), 1023 (s); δ_H (300 MHz, $CDCl_3$) 7.44-7.25 (12H, m, Ar*H*), 6.91 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 4.97 (1H, d, *J* 11.6, OCH_AH_B-Ph), 4.91 (1H, d, *J* 11.3, $OCH_{A'}H_{B'}$-Ph), 4.86 (1H, d, *J* 11.3, $OCH_{A'}H_{B'}$-Ph), 4.73 (1H, d, *J* 11.6, OCH_AH_B-Ph), 4.65 (1H, d, *J* 11.3, OCH_AH_B-$C_6H_4OCH_3$), 4.53 (1H, d, *J* 11.3, OCH_AH_B-$C_6H_4OCH_3$), 4.08 (1H, dd, *J* 2.6, 2.6, inositol ring, H-2), 4.01 (1H, ddd, *J* 9.3, 9.3, 2.1, inositol ring, H-4), 3.84 (3H, s, OCH_2-$C_6H_4OCH_3$), 3.70 (1H, dd, *J* 9.3, 9.3, inositol ring, H-6), 3.55 (1H, ddd, *J* 9.3, 6.5, 2.6, inositol ring, H-1), 3.50 (1H, ddd, *J* 9.3, 9.3, 2.2, inositol ring, H-5), 3.30 (1H, dd, *J* 9.3, 2.6, inositol ring, H-3), 2.62 (1H, d, *J* 2.2, 5-O*H*), 2.59 (1H, d, *J* 2.1, 4-O*H*), 2.36 (1H, d, *J* 6.5, 1-O*H*); δ_C (125 MHz; $CDCl_3$), 159.5 (PMB – Ar*C*), 138.53 (Ar*C*), 138.48 (Ar*C*), 129.6 (PMB – Ar*C*), 129.5 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.7 (Ar*C*H), 114.0 (PMB – Ar*C*H), 81.7 (inositol ring, C-6), 79.8 (inositol ring, C-3), 76.3 (inositol ring, C-2), 75.0 (OCH_2-Ph), 74.8 (inositol ring, C-5), 74.7 (OCH_2-Ph), 72.5 (inositol ring, C-1), 72.4 (inositol ring, C-4), 72.1 ($OCH_2C_6H_4OCH_3$), 55.3 ($OCH_2C_6H_4OCH_3$); *m/z* (ES^+) [Found: $(M+Na)^+$

503.2050. $C_{28}H_{32}O_7Na$ requires M^+, 503.2046]; *m/z* (ES^+) 503 ($[M+Na]^+$, 100%); Anal. Calcd. For $C_{28}H_{32}O_7$: C, 70.0, H, 6.7; Found: C, 70.0, H, 6.7.

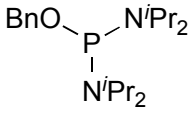
Method 2

(-)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol **139** (540 mg, 1.04 mmol, 1 eq) was dissolved in AcOH (30 mL), with stirring at room temperature $Pd(PPh_3)_4$ (360 mg, 0.31 mmol, 0.3 eq) was added and the reaction monitored by TLC analysis. After 18 h $Pd(PPh_3)_4$ (720 mg, 0.62 mmol, 0.6 eq) was added and the reaction stirred at room temperature for a further 24 h. The reaction was adjudged complete by TLC analysis and quenched by the cautious addition of Na_2CO_3 (s) until pH 7 was achieved. The reaction was then extracted with ethyl acetate (3 × 30 mL) and the combined organic phases dried (magnesium sulfate), filtered and the solvent removed under vacuum. The crude material was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (50:50). This gave *(-)-1D-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-myo-inositol* ***(-)-106*** (463 mg, 93% yield) as a colourless solid. The analysis of this material was consistent with that found for the method 1.

Method 3

(-)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol **139** (60 mg, 0.12 mmol, 1 eq) was dissolved in AcOH (3 mL), $Pd(PPh_3)_4$ (40 mg, 35 µmol, 0.3 eq) was added and the reaction heated to 80 °C. After 4 h further $Pd(PPh_3)_4$ (40 mg, 35 µmol, 0.3 eq) was added and the reaction stirred at room temperature for a further 2 h. The reaction was adjudged complete by TLC analysis and cooled to room temperature before being quenched by the cautious addition of Na_2CO_3 (s) until pH 7 was achieved. The reaction was then extracted with ethyl acetate (3 × 5 mL) and the combined organic phases dried (magnesium sulfate), filtered and the solvent removed under vacuum. The crude material was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (50/50). This gave *(-)-1D-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-myo-inositol* ***(-)-106*** (49 mg, 89% yield) as a colourless solid. The analysis of this material was consistent with that found for the method 1.

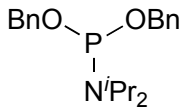
Benzyloxy bis(*N*,*N*-diisopropylamino)phosphine 141



141

Phosphorus trichloride (28 g, 18 mL, 206 mmol, 1 eq) was dissolved in dry diethyl ether (200 mL) under a nitrogen atmosphere. To this solution was added dry pyridine (16 g, 17 mL, 206 mmol, 1 eq) and the mixture cooled to -78 °C. A solution of BnOH (22 g, 21 mL, 206 mmol, 1 eq) in dry diethyl ether (150 mL) was added dropwise over 1 h and the reaction warmed to room temperature and stirred for 1.5 h. The solid formed was removed by Schlenk filtration under nitrogen and washed with diethyl ether. The filtrate was cooled to -10 °C and dry diisopropyl amine (86 g, 118 mL, 845 mmol, 4.1 eq) added over 15 minutes before being warmed to room temperature and stirred for 20 h. The solid formed was removed by Schlenk filtration under a nitrogen atmosphere and washed with diethyl ether. The filtrate was concentrated under reduced pressure to give benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **141** (56 g, 81% yield) as a pale yellow oil. δ_H (300 MHz, $CDCl_3$) 7.43-7.31 (5H, m, Ar*H*), 4.68 (2H, d, *J* 7.3, OC*H*$_2$Ph), 3.68-3.51 [4H, m, N*H*(CH$_3$)$_2$], 1.21 (24H, dd, *J* 6.7, 3.4, NCH(C*H*$_3$)$_2$]; δ_p (121 MHz; $CDCl_3$) 124.6. These data are in good agreement with the literature values.[125]

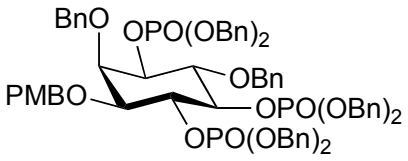
Bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine 142



142

Benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **141** (5 g, 14.8 mmol, 1 eq) and 1*H*-tetrazole (0.43 M solution in acetonitrile, 13 mL, 5.91 mmol, 0.4 eq) were dissolved in dry CH_2Cl_2 (50 mL) under a nitrogen atmosphere. Benzyl alcohol (1.6 g, 1.5 mL, 14.8 mmol, 1 eq) was added at RT, as a solution in dry CH_2Cl_2 (25 mL), dropwise over 45 minutes. The reaction was stirred at RT and TLC analysis indicated the reaction had gone to completion after 3 h. The reaction was concentrated under reduced pressure and the crude material purified by silica gel column chromatography, eluting with triethyl amine, ethyl acetate and petroleum ether (5:15:80). This gave bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** (4.5 g, 88% yield) as a colourless oil. δ_H (300 MHz, $CDCl_3$) 7.43-7.27 (10H, m, Ar*H*), 4.84 (2H, dd, *J* 12.7, $^3J_{HP}$ 8.2, 1 × $POCH_AH_B$-Ph and 1 × $POCH_{A'}H_{B'}$-Ph), 3.85-3.68 [2H, m, $NCH(CH_3)_2$], 1.21 (12H, d, *J* 6.8, $NCH(CH_3)_2$]; δ_p (121 MHz; $CDCl_3$) 147.8. These data are in good agreement with the literature values.[126]

(-)-1D-2,6-bis-*O*-Benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) (-)-107

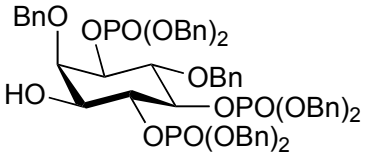


(-)-107

Bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **(-)-142** (406 mg, 1.18 mmol, 6 eq) was stirred under a nitrogen atomosphere with 1*H*-tetrazole (0.43 M solution in acetonitrile, 2.7 mL, 1.18 mmol, 6 eq) for 20 minutes. A solution of (+)-1D-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-*myo*-inositol **(+)-106** (94 mg, 0.2 mmol, 1 eq) in dry CH_2Cl_2 (10 mL) was added *via* a cannular and the reaction left to stir at room temperature overnight. Further bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (135 mg, 0.39 mmol, 2 eq) and 1*H*-tetrazole (0.43M solution in acetonitrile, 900 µL, 0.39 mmol, 2 eq) were added and the reaction stirred at RT for 2 h. The reaction was then cooled to -78 °C and 3-chloroperoxybenzoic acid (60% w/w, 451 mg, 1.57 mmol, 8 eq) added before the solution was warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 10 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3× 10 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (50:50 then 80:20) to give *(-)-1D-2,6-bis-O-benzyl-3-O-(4-methoxy)benzyl-myo-inositol 1,4,5-tris(dibenzylphosphate)* ***(-)-107*** (220 mg, 89% yield) as a gum: R_f 0.48 (ethyl acetate); $[\alpha]_D^{25}$ = -2.8 (*c* 1.00 in $CHCl_3$); υ_{max} (*KBr disc*)/cm^{-1} 3089 (m), 3064 (m), 2957 (m), 2934 (m), 1956 (w), 1888 (w), 1812 (w), 1613 (w), 1514 (m), 1455 (m), 1273 (s), 1016 (s); δ_H (300 MHz, $CDCl_3$) 7.47-7.09 (40H, m, Ar*H*), 7.03-6.97 (2H, m, Ar*H*), 6.81 (2H, d, *J* 8.7, $OCH_2C_6H_4OCH_3$), 5.14-4.64 (17H, m, 6 × $POCH_2$-Ph + 2 × OCH_2-Ph + 1 ×

inositol ring, H-4), 4.59 (1H, ddd, *J* 9.5, 9.5, $^{3}J_{HP}$ 9.5, inositol ring, H-5), 4.51 (1H, d, *J* 11.1, OC*H*$_A$H$_B$C$_6$H$_4$OCH$_3$), 4.45 (1H, d, *J* 11.1, OCH$_A$*H*$_B$C$_6$H$_4$OCH$_3$), 4.38 (1H, dd, *J* 2.1, 2.1, inositol ring, H-2), 4.31 (1H, ddd, *J* 9.5, 2.1, $^{3}J_{HP}$ 7.4, inositol ring, H-1), 4.15 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.78 (3H, s, OCH$_2$C$_6$H$_4$OC*H*$_3$), 3.49 (1H, dd, *J* 9.9, 2.1, inositol ring, H-3); δ_C (125 MHz; CDCl$_3$), 159.6 (PMB – Ar*C*), 138.7 (Ar*C*), 138.7 (Ar*C*), 136.7 (d, $^{3}J_{CP}$ 7.8, Ar*C*), 136.6 (d, $^{3}J_{CP}$ 7.5, Ar*C*), 136.6 (d, $^{3}J_{CP}$ 8.0, Ar*C*), 136.3 (d, $^{3}J_{CP}$ 7.1, Ar*C*), 136.0 (d, $^{3}J_{CP}$ 7.2, Ar*C*), 136.0 (d, $^{3}J_{CP}$ 6.9, Ar*C*), 129.9 (Ar*C*H), 129.8 (PMB – Ar*C*), 129.0 (Ar*C*H), 129.0 (Ar*C*H), 128.7 (Ar*C*H), 128.7 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 127.6 (Ar*C*H), 114.2 (PMB – Ar*C*H), 79.6-79.4 (m, inositol ring, C-5), 78.6-78.2 (m, inositol ring, C-1, 4 and 6), 77.9 (inositol ring, C-3), 75.7 (O*C*H$_2$-Ph), 75.6 (inositol ring, C-2), 75.0 (O*C*H$_2$-Ph), 72.4 (O*C*H$_2$C$_6$H$_4$OCH$_3$), 70.0-69.8 (3 × PO*C*H$_2$-Ph), 69.7 (d, $^{2}J_{CP}$ 5.7, PO*C*H$_2$-Ph), 69.6 (d, $^{2}J_{CP}$ 4.9, PO*C*H$_2$-Ph), 69.5 (d, $^{2}J_{CP}$ 4.9, PO*C*H$_2$-Ph), 55.6 (OCH$_2$C$_6$H$_4$O*C*H$_3$); δ_p (121 MHz; CDCl$_3$) -1.63, -1.83, -2.04; *m/z* (ES$^+$) [Found: (M+Na)$^+$ 1283.3835. C$_{70}$H$_{71}$O$_{16}$P$_3$Na requires *M*$^+$, 1283.3847]; *m/z* (ES+) 1283 ([M+Na]$^+$, 100%); Anal. Calcd. For C$_{70}$H$_{71}$O$_{16}$P$_3$: C, 66.7, H, 5.7; Found: C, 66.8, H, 5.6.

(-)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) (-)-108

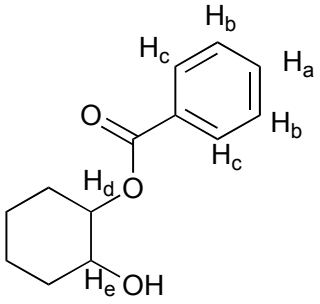


(-)-108

(-)-1D-2,6-bis-*O*-Benzyl-3-*O*-(4-methoxy)benzyl-myo-inositol 1,4,5-tris(dibenzylphosphate) **(-)-107** (53 mg, 42 μmol, 1 eq) was dissolved in CH_2Cl_2 (10 mL). To this solution was added TMSCl (14 mg, 16 μl, 126 μmol, 3 eq) followed by anisole (7 mg, 7 μl, 63 μmol, 1.5 eq). The reaction stirred at RT for 5

min before the addition of $SnCl_2 \cdot 2H_2O$ (catalytic, 0.1 eq) and monitored by TLC analysis. The reaction was adjudged complete after 3 h. The reaction was diluted with H_2O (10 mL), the layers were separated and the aqueous layer further extracted with CH_2Cl_2 (3× 5 mL). The combined organic layers were then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (50:50 then 60:40, 70:30, 80:20) to give *(-)-1D-2,6-bis-O-benzyl-myo-inositol 1,4,5-tris(dibenzylphosphate)* ***(-)-108*** (35 mg, 77% yield) as a colourless waxy solid: R_f 0.37 (3% MeOH in CH_2Cl_2); $[\alpha]_D^{25}$ = -23.8 (*c* 1.00 in $CHCl_3$); υ_{max} (*KBr disc*)/cm^{-1} 3330 (s, broad), 3089 (m), 3066 (m), 2925 (s), 1497 (m), 1455 (m), 1260 (s), 1016 (s); δ_H (400 MHz, $CDCl_3$) 7.44-7.12 (36H, m, Ar*H*), 7.11-7.07 (2H, m, Ar*H*), 7.00-6.96 (2H, m, Ar*H*), 5.17-4.74 (15H, m, POCH_AH_B-Ph + 2 × OCH_AH_B-Ph), 4.74-4.62 (2H, m, POCH_AH_B-Ph + inositol ring, H-4), 4.51 (1H, ddd, $^3J_{HP}$ 9.5, *J* 9.3, 9.3, inositol ring, H-5), 4.34 (1H, ddd, *J* 9.5, 2.5, $^3J_{HP}$ 7.6, inositol ring, H-1), 4.29 (1H, dd, *J* 2.5, 2.5, inositol ring, H-2), 4.08 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.72 (1H, ddd, *J* 9.5, 3.7, 2.5, inositol ring, H-3); δ_C (75 MHz; $CDCl_3$), 139.0 (Ar*C*), 138.5 (Ar*C*), 129.0 (Ar*C*H), 128.9 (Ar*C*H), 128.8 (Ar*C*H), 128.7 (Ar*C*H), 128.5 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 127.7 (Ar*C*H), 127.7 (Ar*C*H), 80.8 (dd, $^2J_{CP}$ 4.8, $^3J_{CP}$ 4.8, inositol ring, C-4), 79.1 (inositol ring, C-2), 79.1-78.9 (m, inositol ring, C-5), 78.3 (d, $^3J_{CP}$ 8.2, inositol ring, C-6), 78.1 (d, $^2J_{CP}$ 5.9, inositol ring, C-1), 76.4 (O*C*H_2-Ph), 75.0 (O*C*H_2-Ph), 71.7 (inositol ring, C-3), 70.7 (d, $^2J_{CP}$ 5.7, PO*C*H_2-Ph), 70.6 (d, $^2J_{CP}$ 5.7, PO*C*H_2-Ph), 69.9 (d, $^2J_{CP}$ 5.6, PO*C*H_2-Ph), 69.8 (d, $^2J_{CP}$ 5.6, PO*C*H_2-Ph), 69.7 (d, $^2J_{CP}$ 5.7, PO*C*H_2-Ph), 69.6 (d, $^2J_{CP}$ 5.0, PO*C*H_2-Ph); δ_p (121 MHz; $CDCl_3$) 0.8, -1.3, -1.6; *m/z* (ES^+) [Found: $(M+H)^+$ 1141.3447. $C_{62}H_{64}O_{15}P_3$ requires M^+, 1141.3453]; *m/z* (ES+) 1141 ($[M+H]^+$, 100%); Anal. Calcd. For $C_{62}H_{64}O_{15}P_3$: C, 66.7, H, 5.7; Found: C, 66.6, H, 5.8. Further reactions on a large scale using the conditions described gave variable yield 19-40%.

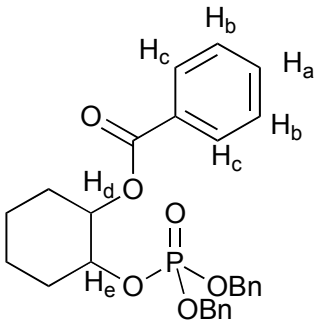
(±)-1-*O*-Benzoyl-*trans*-cyclohexane-1,2-diol 147



147

Dibutyltin oxide (12.0 g, 51.69 mmol, 3 eq) was added to a solution of (±)-*trans*-cyclohexane-1,2-diol **146** (2.0 g, 17.23 mmol, 1 eq) in dry toluene (50 mL). Dean and Stark apparatus was attached and the mixture was heated under reflux overnight to pre-form the tin acetal complex. The mixture was cooled to 0 °C and benzoyl chloride (3.6 mg, 3.0 mL, 25.84 mmol, 1.5 eq) was added. The reaction was stirred at RT and adjudged to be complete by TLC analysis after 6 h. The volatile components were removed under vacuum and the residue partitioned between ethyl acetate (50 mL) and water (50 mL), the layers were separated and the aqueous phase extracted with ethyl acetate (3 × 30 mL). The combined organics were dried (magnesium sulfate), filtered and the solvent removed under vacuum. The crude material was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (20:80, 30:70 then 50:50). This gave (±)-1-*O*-benzoyl-*trans*-cyclohexane-1,2-diol **147** (1.8 g, 47% yield) as a colourless crystalline solid: R_f 0.48 (petroleum ether / ethyl acetate 60:40); mp 98-100 °C (hexane/ethyl acetate); δ_H (300 MHz, $CDCl_3$) 8.14-8.08 (2H, m, H_c), 7.64-7.57 (1H, m, H_a), 7.52-7.45 (2H, m, H_b), 4.94-4.84 (1H, m, H_d), 3.83-3.73 (1H, m, H_e), 2.38 (1H, br s, -O*H*), 2.25-2.09 (2H, m, *cyclohexane ring*), 1.88-1.72 (2H, m, *cyclohexane ring*), 1.57-1.28 (4H, m, *cyclohexane ring*). These data are in agreement with the literature values.[127]

(±)-1-*O*-Dibenzylphosphate-2-*O*-benzoyl-2-*trans*-cyclohexane-1,2-diol 148

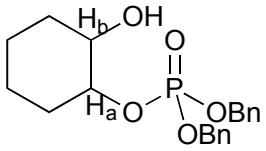


148

Bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (314 mg, 909 μmol, 2 eq) was stirred under a nitrogen atomosphere with 1*H*-tetrazole (0.43 M solution in acetonitrile, 2.1 mL, 909 μmol, 2 eq) for 20 minutes. A solution of (±)-1-*O*-benzoyl-*trans*-cyclohexane-1,2-diol **147** (100 mg, 454 μmol, 1 eq) in dry CH_2Cl_2 (10 mL) was added *via* a cannular and the reaction left to stir at room temperature overnight. Further bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (157 mg, 454 μmol, 1 eq) and 1*H*-tetrazole (0.43 M solution in acetonitrile, 1 mL, 454 μmol, 1 eq) were added and the reaction stirred at RT for 2 h. The reaction was then cooled to -78 °C and 3-chloroperoxybenzoic acid (60% w/w, 393 mg, 1363 μmol, 3 eq) added before being warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 10 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3× 10 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (20:80 then 50:50) to give (±)-1-*O*-benzoyl-2-*O*-dibenzylphosphate-*trans*-cyclohexane-1,2-diol **148** (123 mg, 56% yield) as a colourless gum: R_f 0.42 (hexane / ethyl acetate 60:40); δ_H (300 MHz, $CDCl_3$) 8.03-7.94 (2H, m, H_c), 7.48-7.38 (1H, m, H_a), 7.33-7.07 (10H, m, benzyl Ar*H*), 7.01-6.90 (2H, m, H_b), 5.03-4.92 (1H, m, H_d), 4.92-4.82 (2H, m, benzylic CH_2), 4.81-4.65 (2H, m, benzylic CH_2), 4.56-4.43 (1H, m, H_e), 2.10-2.04

(2H, m, *cyclohexane ring*), 1.76-1.60 (2H, m, *cyclohexane ring*), 1.60-1.13 (4H, m, *cyclohexane ring*); δ_p (121 MHz; $CDCl_3$) 1.9.

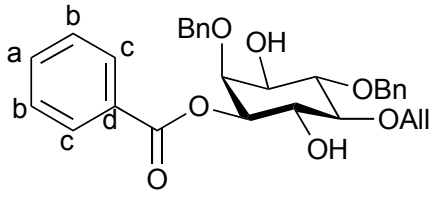
(±)-1-*O*-Dibenzylphosphate-*trans*-cyclohexane-1,2-diol 149



149

(±)-1-*O*-Benzoyl-2-*O*-dibenzylphosphate-*trans*-cyclohexane-1,2-diol **148** (76 mg, 0.16 mmol, 1 eq) was dissolved in dry CH_2Cl_2 (3.5 mL) under a nitrogen atmosphere and cooled to -78 °C. With vigorous stirring diisobutylaluminium hydride (868 µL of a 1 M solution in hexane, 0.87 mmol, 5.5 eq) was added slowly dropwise. After 15 minutes TLC analysis indicated the full consumption of starting material. The DIBALH was quenched with the slow addition of methanol (5 ml) (CAUTION: copious effervescence and a slight exotherm is observed). The reaction mixture was warmed to RT and treated with $Na_2SO_4 \cdot 10H_2O$ (Note: A thick gel formed, this was broken up with the addition of more $Na_2SO_4 \cdot 10H_2O$). The solid was removed by filtration through Celite® and washed with ethyl acetate. The filtrate was concentrated under vacuum and the crude material purified by silica gel column chromatography eluting with ethyl acetate and hexane (40:60) to give (±)-1-*O*-dibenzylphosphate-*trans*-cyclohexane-1,2-diol **149** (52 mg, 87% yield) as a colourless solid: R_f 0.34 (ethyl acetate/hexane 4:6); δ_H (300 MHz, $CDCl_3$) 7.51-7.02 (10H, m, benzyl Ar*H*), 5.20-4.78 (4H, m, benzylic CH_2), 4.02-3.92 (1H, m, H_a), 3.50-3.39 (1H, m, H_b), 3.34 (1H, br s –O*H*), 2.02-1.87 (2H, m, *cyclohexane ring*), 1.66-1.50 (2H, m, *cyclohexane ring*), 1.38-1.02 (4H, m, *cyclohexane ring*); δ_p (121 MHz; $CDCl_3$) -0.3.

(±)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-benzoyl-*myo*-inositol 150

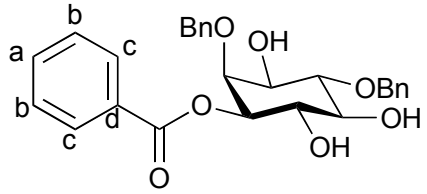


150

Dibutyltin oxide (465 mg, 1.87 mmol, 1.5 eq) was added to a solution of (±)-1D-5-*O*-allyl-2,6-bis-*O*-benzyl-*myo*-inositol **116** (500 mg, 1.25 mmol, 1 eq) in dry toluene (15 mL). A Soxhlet apparatus containing 3 Å molecular sieves was attached and the mixture was heated under reflux overnight to pre-form the tin acetal complex. The solution was cooled to RT and 4 Å molecular sieves added. The mixture was then cooled to -5 °C and benzoyl chloride (351 mg, 290 µL, 2.50 mmol, 2 eq) was added. The reaction was allowed to stir at RT for 3 h. The molecular sieves were removed by filtration and the volatile components were removed under vacuum and the residue partitioned between ethyl acetate (30 mL) and water (30 mL), the layers were separated and the aqueous phase extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (10:90 then 30:70) to give *(±)-1D-5-O-allyl-2,6-bis-O-benzyl-3-O-benzoyl-myo-inositol* **150** (366 mg, 60% yield) as a colourless gum: R_f 0.60 (hexane / ethyl acetate 50:50); υ_{max} (*KBr disc*)/cm^{-1} 3519 (s, broad), 3385 (s, broad), 3089 (m), 3066 (m), 3032 (m), 2922 (s), 1712 (s), 1273 (s), 1112 (s); δ_H (400 MHz, $CDCl_3$) 8.07 (2H, d, *J* 8.2, benzoyl Ar*H*, H_c), 7.60 (1H, t, *J* 7.4, benzoyl Ar*H*, H_a), 7.46 (2H, dd, *J* 8.2, 7.4, benzoyl Ar*H*, H_b), 7.43-7.24 (10H, m, benzyl Ar*H*), 5.99 (1H, dddd, *J* 17.1, 10.8, 5.8, 5.6, CH_X=CH$_Y$H$_Z$), 5.32 (1H, ddd, *J* 17.1, 1.9, 1.9, CH$_X$=CH_YH$_Z$), 5.20 (1H, ddd, *J* 10.8, 1.3, 1.3, CH$_X$=CH$_Y$$H_Z$), 5.05 (1H, dd, *J* 9.5, 2.5, inositol ring, H-3), 4.93 (1H, d, *J* 11.1, OCH_AH$_B$-Ph), 4.80 (1H, d, *J*

11.1, OCH_AH_B-Ph), 4.80 (1H, d, *J* 11.6, $OCH_{A'}H_{B'}$-Ph), 4.71 (1H, d, *J* 11.6, $OCH_{A'}H_{B'}$-Ph), 4.43 (1H, dddd, *J* 12.6, 5.6, 1.9, 1.3, OCH_VH_W-$CH=CH_2$), 4.35 (1H, dddd, *J* 12.6, 5.8, 1.9, 1.3, OCH_VH_W-$CH=CH_2$), 4.27 (1H, ddd, *J* 9.5, 9.5, 3.3, inositol ring, H-4), 4.18 (1H, dd, *J* 2.5, 2.5, inositol ring, H-2), 3.82 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.71 (1H, ddd, *J* 9.5, 6.0, 2.5, inositol ring, H-1), 3.36 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5), 2.41 (1H, d, *J* 3.3, 4-O*H*), 2.28 (1H, d, *J* 6.0, 1-O*H*); δ_C (125 MHz; $CDCl_3$), 166.1 (*C*=O), 138.4 (Ar*C*), 138.2 (Ar*C*), 134.9 (OCH_2-*C*$H=CH_2$), 133.4 (benzoyl Ar*C*H, C_a), 129.8 (benzoyl Ar*C*H, C_b), 129.6 (benzoyl Ar*C*, C_d), 128.6 (benzoyl Ar*C*H, C_c), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.1 (Ar*C*H), 127.9 (Ar*C*H), 127.7 (Ar*C*H), 127.6 (Ar*C*H), 117.2 (OCH_2-$CH=CH_2$), 83.0 (inositol ring, C-5), 81.6 (inositol ring, C-6), 77.8 (inositol ring, C-2), 75.5 (OCH_2-Ph), 75.3 (OCH_2-Ph), 74.3 (OCH_2-$CH=CH_2$), 74.2 (inositol ring, C-3), 72.3 (inositol ring, C-1), 71.6 (inositol ring, C-4); *m/z* (ES^+) [Found: $(M+Na)^+$ 527.2035. $C_{30}H_{32}O_7Na$ requires M^+, 527.2040]; *m/z* (ES^+) 606 ($[M+HNEt_3]^+$, 100%), 527 ($[M+Na]^+$, 40%); Anal. Calcd. For $C_{30}H_{32}O_7$: C, 71.4, H, 6.4; Found: C, 71.4, H, 6.3.

(±)-1D-2,6-bis-*O*-Benzyl-3-*O*-benzoyl-*myo*-inositol 151

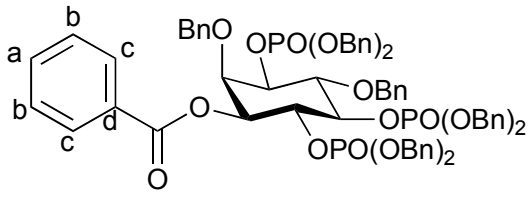


151

(±)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-benzoyl-*myo*-inositol **150** (366 mg, 0.73 mmol, 1 eq) was dissolved in AcOH (20 mL), with stirring at room temperature $Pd(PPh_3)_4$ (838 mg, 0.73 mmol, 1 eq) was added and the reaction monitored by TLC analysis. After 18 h the reaction was adjudged complete by TLC analysis. The reaction mixture was concentrated by the azeotropic removal of AcOH with cyclohexane. The crude material was purified by silica gel column

chromatography eluting with ethyl acetate and petroleum ether (50:50). This gave *(±)-1D-2,6-bis-O-benzyl-3-O-benzoyl-myo-inositol* **151** (290 mg, 86% yield) as a colourless solid: R_f 0.44 (ethyl acetate/petroleum ether 60:40); υ_{max} (*KBr disc*)/cm^{-1} 3429 (s broad), 3063 (m), 3031 (m), 2927 (m), 1721 (s), 1317 (s), 1104 (s); δ_H (300 MHz, $CDCl_3$) 8.08 (2H, d, *J* 7.0 benzoyl Ar*H,* H_c), 7.61 (1H, t, *J* 7.4, benzoyl Ar*H*, H_a), 7.46 (2H, dd, *J* 7.4, 7.0, benzoyl Ar*H*, H_b), 7.43-7.25 (10H, m, benzyl Ar*H*), 5.03 (1H, dd, *J* 9.7, 2.4, inositol ring, H-3), 4.92 (1H, d, *J* 11.7, OC$H_A$$H_B$-Ph), 4.88 (1H, d, *J* 11.7, OC$H_A$$H_B$-Ph), 4.83 (1H, d, *J* 11.6, OC$H_{A'}$$H_{B'}$-Ph), 4.72 (1H, d, *J* 11.6, OC$H_{A'}$$H_{B'}$-Ph), 4.20 (1H, ddd, *J* 9.7, 9.7, 3.9, inositol ring, H-4), 4.20 (1H, dd, J 2.4, 2.4, inositol ring, H-2), 3.81-3.69 (2H, m, 2 × inositol ring, H-1 and H-6), 3.56 (1H, ddd, J 9.7, 9.7, 2.6, inositol ring, H-5), 2.96 (1H, d, *J* 2.6, 5-O*H*), 2.75 (1H, d, *J* 3.9, 4-O*H*), 2.41 (1H, d, *J* 6.0, 1-O*H*); δ_C (125 MHz; $CDCl_3$), 166.6 (*C*=O), 139.5 (Ar*C*), 139.0 (Ar*C*), 133.4 (benzoyl Ar*C*H, C_a), 130.3 (benzoyl Ar*C*, C_d), 129.9 (benzoyl Ar*C*H, C_c), 128.5 (benzoyl Ar*C*H, C_b), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 127.8 (Ar*C*H), 127.5 (Ar*C*H), 82.2 (inositol ring, C-6), 79.2 (inositol ring, C-2), 75.7 (inositol ring, C-5), 75.3 (O*C*H_2-Ph), 75.1 (inositol ring, C-3), 75.1 (O*C*H_2-Ph), 72.4 (inositol ring, C-1), 71.7 (inositol ring, C-4); *m/z* (ES^+) [Found: $(M+Na)^+$ 487.1725. $C_{27}H_{28}O_7Na$ requires M^+, 487.1727]; *m/z* (ES^+) 566 ($[M +HNEt_3]^+$, 100%), 487 ($[M+Na]^+$, 20%); Anal. Calcd. For $C_{27}H_{28}O_7$: C, 69.8, H, 6.1; Found: C, 70.0, H, 6.1.

(±)-1D-2,6-bis-*O*-Benzyl-3-*O*-benzoyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) 145



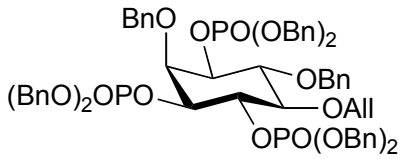
145

Bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (228 mg, 0.66 mmol, 6 eq) was stirred under a nitrogen atomosphere with 1*H*-tetrazole (0.43 M solution in acetonitrile, 1.5 mL, 0.66 mmol, 6 eq) for 20 minutes. A solution of (-)-1D-2,6-bis-*O*-benzyl-3-*O*-benzoyl-*myo*-inositol **151** (51 mg, 0.11 mmol, 1 eq) in dry CH_2Cl_2 (5 mL) was added to the phosphoramidite and tetrazole solution *via* a cannular and the reaction left to stir at room temperature overnight. More bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (76 mg, 0.22 mmol, 2 eq) and 1*H*-tetrazole (0.43M solution in acetonitrile, 500 µL, 0.22 mmol, 2 eq) were added and the reaction mixture stirred at RT for 2 h. The reaction was then cooled to -78 °C and 3-chloroperoxybenzoic acid (70% w/w, 152 mg, 0.88 mmol, 8 eq) added before being warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 5 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (50:50 then 80:20) to give *(±)-1D-2,6-bis-O-benzyl-3-O-benzoyl-myo-inositol 1,4,5-tris(dibenzylphosphate)* **145** (96 mg, 70% yield) as a colourless gum: R_f 0.56 (ethyl acetate); ν_{max} (*NaCl plate*)/cm^{-1} 3090 (m), 3064 (m), 2955 (m), 2894 (m), 1956 (w), 1885 (w), 1814 (w), 1721 (s), 1314 (s), 1013 (s); δ_H (500 MHz, $CDCl_3$) 8.07 (2H, d, *J* 7.5, benzoyl Ar*H*, H_c), 7.53 (1H, t, *J* 7.4, benzoyl Ar*H*, H_a), 7.42-7.39 (2H, m, ArH), 7.37 (2H, dd, *J* 7.5, 7.4, benzoyl Ar*H*,

H_b), 7.30-7.15 (28H, m, benzyl Ar*H*), 7.14-7.08 (4H, m, Ar*H*), 7.08-7.03 (2H, m, Ar*H*), 7.03-6.98 (2H, m, Ar*H*), 6.84-6.80 (2H, m, Ar*H*), 5.26 (1H, ddd, *J* 9.5, 9.5, $^3J_{HP}$ 9.5, inositol ring, H-4), 5.16 (1H, dd, *J* 9.5, 2.4, inositol ring, H-3), 5.02 (1H, dd, *J* 11.6, $^3J_{HP}$ 3.4, POCH_XH$_Y$-Ph), 5.00 (1H, dd, *J* 11.1, $^3J_{HP}$ 4.1, POC$H_{X'}$H$_{Y'}$-Ph), 4.96-4.77 (9H, m, POCH_2-Ph + OCH_2-Ph), 4.73-4.65 (4H, m, POCH_2-Ph + OCH_2-Ph), 4.62 (1H, ddd, *J* 9.5, 9.5, $^3J_{HP}$ 9.5, inositol ring, H-5), 4.50-4.42 (2H, m, POCH_XH$_Y$-Ph + inositol ring, H-1), 4.40 (1H, dd, *J* 2.4, 2.4, inositol ring, H-2), 4.19 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6); δ_C (125 MHz; $CDCl_3$), 165.1 (*C*=O), 138.1 (Ar*C*), 137.6 (Ar*C*), 135.9 (d, $^3J_{CP}$ 7.4, Ar*C*), 135.8 (d, $^3J_{CP}$ 8.0, Ar*C*), 135.8 (d, $^3J_{CP}$ 7.3, Ar*C*), 135.5 (d, $^3J_{CP}$ 6.6, Ar*C*), 135.4 (d, $^3J_{CP}$ 6.6, Ar*C*), 135.3 (d, $^3J_{CP}$ 7.2, Ar*C*), 133.3 (benzoyl Ar*C*H, C_a), 130.2 (benzoyl Ar*C*H, C_c), 129.1 (benzoyl Ar*C*, C_d), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.3 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.6 (Ar*C*H), 127.4 (Ar*C*H), 127.3 (Ar*C*H), 127.2 (Ar*C*H), 78.7 (dd, $^2J_{CP}$ 7.1, $^3J_{CP}$ 7.1, inositol ring, C-5), 77.8 (d, $^2J_{CP}$ 7.5, inositol ring, C-1), 77.5 (d, $^3J_{CP}$ 6.2, inositol ring, C-6), 76.5 (dd, $^2J_{CP}$ 5.2, $^3J_{CP}$ 5.2, inositol ring, C-4), 76.2 (inositol ring, C-2), 75.6 (O*C*H$_2$-Ph), 74.7 (O*C*H$_2$-Ph), 71.5 (inositol ring, C-3), 69.6 (d, $^2J_{CP}$ 5.5, PO*C*H$_2$-Ph), 69.5 (d, $^2J_{CP}$ 5.9, PO*C*H$_2$-Ph), 69.4 (d, $^2J_{CP}$ 6.0, PO*C*H$_2$-Ph), 69.4 (d, $^2J_{CP}$ 6.0, PO*C*H$_2$-Ph), 69.3 (d, $^2J_{CP}$ 5.0, PO*C*H$_2$-Ph), 68.9 (d, $^2J_{CP}$ 5.4, PO*C*H$_2$-Ph); δ_p (161 MHz; $CDCl_3$) -1.7, -1.8, -2.0; *m/z* (ES^+) [Found: $(M+Na)^+$ 1267.35 (100.0%), 1238.36 (78.0%), 1269.36 (32.5%), 1270.36 (7.3%), 1271.36 (1.2%). $C_{69}H_{67}O_{16}P_3Na$ calculated M^+, 1267.35 (100.0%), 1268.36 (76.0%), 1269.36 (31.8%), 1270.36 (9.5%), 1271.37 (2.3%)]; *m/z* (ES^+) 1346 ($[M +HNEt_3]^+$, 100%), 1267 ($[M+Na]^+$, 40%); Anal. Calcd. For $C_{69}H_{67}O_{16}P_3$: C, 66.6, H, 5.4; Found: C, 66.7, H, 5.4.

8.4. Synthesis of 5-O-position compounds

(+)-1D-2,6-bis-*O*-Benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 152

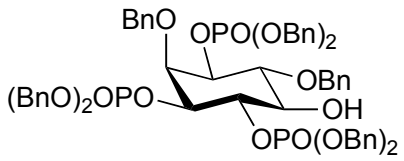


(+)-152

To a solution of the triol **138** (1.0 g, 2.5 mmol, 1 eq) in dry CH_2Cl_2 (50 mL) under a nitrogen atomosphere was added 1*H*-tetrazole (0.43M solution in acetonitrile, 33 mL, 15.0 mmol, 6 eq) followed by bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** (5.2 g, 15.0 mmol, 6 eq) the reaction left to stir at RT overnight. Further 1*H*-tetrazole (0.43M solution in acetonitrile, 11.1 mL, 5.0 mmol, 2 eq) followed by bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** (1.73 g, 5.0 mmol, 2 eq) was added and the reaction stirred at RT for 2 h. Further 1*H*-tetrazole (0.43 M solution in acetonitrile, 11.1 mL, 5.0 mmol, 2 eq) followed by bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine **142** (1.73 g, 5.0 mmol, 2 eq) was added and the reaction mixture stirred at RT for another 2 h. The reaction mixture was then cooled to -78 °C and 3-chloroperoxybenzoic acid (80% w/w, 5.5 g, 25.0 mmol, 10 eq) added before being warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 50 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with Et_2O (100%) then ethyl acetate and hexane (60:40, 70:30, 80:20) to give *(-)-1D-2,6-bis-O-benzyl-myo-inositol 1-O-benzyl-O-methylphosphate-3,4,5-tris(dibenzylphosphate)* **152** (2.5 g, 86% yield) as a colourless gum: R_f 0.37 (4% MeOH in $CHCl_3$); $[\alpha]_D^{25}$= +2.00 (*c* 1.00 in $CHCl_3$); υ_{max} (*NaCl disc*)/cm^{-1} 3090 (w), 3064 (m), 3033 (m), 2961 (m), 2895 (m), 2360

(w), 2340 (w), 1956 (w), 1884 (w), 1814 (w), 1731 (w); δ_H (500 MHz, $CDCl_3$) 7.36-7.14 (40H, m, Ar*H*), 5.85 (1H, dddd, *J* 17.0, 11.3, 5.7, 5.7, CH_X=CH$_Y$H$_Z$), 5.16 (1H, dd, *J* 17.0, 1.6, CH$_X$=CH_YH$_Z$), 5.09-4.71 (18H, m, 8 × OC$H_A$$H_B$-Ph + CH$_X$=CH$_Y$$H_Z$ + 1 × inositol ring, H-4), 4.60 (1H, dd, *J* 2.3, 2.3, inositol ring, H-2), 4.33-4.10 (4H, m, OC$H_V$$H_W$-CH=CH$_2$ + 2 × inositol ring, H-1 and H-3), 4.01 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.23 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5); δ_C (125 MHz; $CDCl_3$), 138.3 (Ar*C*), 138.1 (Ar*C*), 136.1 (d, $^3J_{CP}$ 7.8, Ar*C*), 136.0 (d, $^3J_{CP}$ 7.6, Ar*C*), 135.6 (d, $^3J_{CP}$ 6.7, Ar*C*), 135.6 (d, $^3J_{CP}$ 7.0, Ar*C*), 135.5 (d, $^3J_{CP}$ 7.6, Ar*C*), 134.6 (*C*H=CH$_2$), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.4 (Ar*C*H), 128.4 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.5 (Ar*C*H), 127.4 (Ar*C*H), 127.2 (Ar*C*H), 117.0 (CH=*C*H$_2$), 80.1 (inositol ring, C-5), 79.4 (d, $^2J_{CP}$ 6.5, inositol ring, C-6), 77.8 (dd, $^2J_{CP}$ 7.2, $^3J_{CP}$ 7.2, inositol ring, C-4), 77.7 (inositol ring, C-2), 77.4 (d, $^2J_{CP}$ 6.4, inositol ring, C-1), 75.9 (dd, $^2J_{CP}$ 4.7, $^3J_{CP}$ 4.0, inositol ring, C-3), 75.8 (O*C*H$_2$-Ph), 75.6 (O*C*H$_2$-Ph), 74.5 (O*C*H$_2$-CH=CH$_2$), 69.8 (d, $^2J_{CP}$ 5.7, PO*C*H$_2$-Ph), 69.5 (d, $^2J_{CP}$ 5.2, PO*C*H$_2$-Ph), 69.4 (d, $^2J_{CP}$ 5.7, PO*C*H$_2$-Ph), 69.4 (d, $^2J_{CP}$ 5.5, PO*C*H$_2$-Ph), 69.3 (d, $^2J_{CP}$ 5.1, PO*C*H$_2$-Ph), 69.2 (d, $^2J_{CP}$ 5.2, PO*C*H$_2$-Ph); δ_p (161 MHz; $CDCl_3$) -1.3, -1.5, -2.0; *m/z* (ES^+) [Found: $(M+Na)^+$ 1203.36 (100.0%), 1204.37 (72.8%), 1205.37 (28.8%), 1206.37 (6.6%), 1207.37 (1.0%). $C_{65}H_{67}NaO_{15}P_3$ calculated M^+, 1203.36 (100.0%), 1204.36 (71.6%), 1205.37 (28.4%), 1206.37 (8.1%), 1207.37 (1.8%)]; *m/z* (ES^+) 1193 ($[M-Bn+HNEt_3]^+$, 30%), 1203 ($[M+Na]^+$, 40%), 1193 ($[M+HNEt_3]^+$, 100%); Anal. Calcd. For $C_{65}H_{67}O_{15}P_3$: C, 66.1, H, 5.7; Found: C, 66.2, H, 5.6.

(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 153



(+)-153

Method 1

To a solution of (+)-1D-2,6-bis-*O*-benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **152** (100 mg, 87 µmol, 1 eq) in AcOH (7 mL) under a nitrogen atomosphere was added $Pd(PPh_3)_4$ (60 mg, 52 µmol, 0.6 eq) the reaction left to stir at RT. After 2 h, further $Pd(PPh_3)_4$ (100 mg, 87 µmol, 1 eq) was added and the reaction stirred for a another 3 h. The reaction was concentrated by the azeotropic removal of AcOH with toluene. The resulting residue was purified by silica gel column chromatography, eluting with ethylacetate and petroleum ether (30:70, 70:30, 100:0), then again with ethylacetate and petroleum ether (20:80, 40:60, 50:50, 70:30) and then finally with methanol and chloroform (3:97, 4:96, 5:95). This afforded *(+)-1D-2,6-bis-O-benzyl-myo-inositol 1,3,4-tris(dibenzylphosphate)* **153** (55 mg, 55% yield) as a colourless gum: R_f 0.28 (4% MeOH in $CHCl_3$); $[\alpha]_D^{25}$= +4.37 (*c* 1.26 in $CHCl_3$); υ_{max} (*NaCl disc*)/cm^{-1} 3385 (m), 3090 (m), 3063 (m), 3033 (m), 2956 (m), 1957 (w), 1883 (w), 1814 (w), 1727 (w), 1631 (s), 1606 (s), 1264 (s), 1017 (s); δ_H (500 MHz, $CDCl_3$) 7.43-7.38 (2H, m, Ar*H*), 7.35-7.16 (38H, m, Ar*H*), 5.11-5.02 (4H, m, OCH_AH_B-Ph), 5.02-4.88 (9H, m, OCH_AH_B-Ph), 4.80-4.69 (4H, m, 3 × OCH_AH_B-Ph + 1 × inositol ring, H-4), 4.53 (1H, dd, *J* 2.5, 2.5, inositol ring, H-2), 4.33-4.26 (2H, m, 2 × inositol ring, H-1 and H-3), 3.98 (1H, dd, *J* 9.2, 9.2, inositol ring, H-6), 3.72 (1H, dd, *J* 9.2, 9.2, inositol ring, H-5), 2.1 (1H, br s, O*H*); δ_C (125 MHz; $CDCl_3$), 138.4 (Ar*C*), 138.2 (Ar*C*), 135.7 (d, $^3J_{CP}$ 7.5, Ar*C*), 135.6 (d, $^3J_{CP}$ 7.5, Ar*C*), 135.5 (d, $^3J_{CP}$ 7.6, Ar*C*), 135.4 (d, $^3J_{CP}$ 7.6, Ar*C*), 135.4 (d, $^3J_{CP}$ 6.3, Ar*C*), 128.6 (Ar*C*H), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.4

(Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.7 (Ar*C*H), 127.6 (Ar*C*H), 127.5 (Ar*C*H), 127.4 (Ar*C*H), 127.3 (Ar*C*H), 79.8 (dd, $^{2}J_{CP}$ 6.2, $^{3}J_{CP}$ 6.2, inositol ring, C-4), 79.2 (d, $^{3}J_{CP}$ 6.7, inositol ring, C-6), 78.0 (inositol ring, C-2), 77.0* (inositol ring, C-1), 75.8 (O*C*H_2-Ph), 75.6 (dd, $^{2}J_{CP}$ 6.0, $^{3}J_{CP}$ 6.0, inositol ring, C-3), 75.5 (O*C*H_2-Ph), 74.2 (inositol ring, C-5), 70.1 (d, $^{2}J_{CP}$ 6.1, PO*C*H_2-Ph), 70.0 (d, $^{2}J_{CP}$ 6.0, PO*C*H_2-Ph), 69.7 (d, $^{2}J_{CP}$ 5.6, PO*C*H_2-Ph), 69.5 (d, $^{2}J_{CP}$ 5.4, PO*C*H_2-Ph), 69.3 (d, $^{2}J_{CP}$ 5.5, PO*C*H_2-Ph); δ_p (161 MHz; $CDCl_3$) 1.7, -0.5, -0.5; *m/z* (ES^+) [Found: $(M+Na)^+$ 1163.33 (100.0%), 1164.33 (68.5%), 1165.33 (21.3%), 1166.34 (5.9%), 1167.35 (0.8%). $C_{62}H_{63}NaO_{15}P_3$ calculated M^+, 1163.33 (100.0%), 1164.33 (68.4%), 1165.33 (26.1%), 1166.34 (7.2%), 1167.34 (1.6%)]; *m/z* (ES^+) 1199 ($[M +NH_4{\cdot}MeCN]^+$, 100%), 1163 ($[M+Na]^+$, 5%); Anal. Calcd. For $C_{62}H_{63}O_{15}P_3$: C, 65.3, H, 5.6; Found: C, 65.3, H, 5.5.

*Signal was assigned from the HSQC NMR spectrum, as the solvent peak swamped the signal in the 1D ^{13}C spectrum.

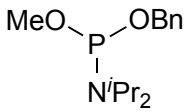
Method 2

To a solution of (+)-1D-2,6-bis-*O*-benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **152** (100 mg, 84 µmol, 1 eq) in AcOH (7 mL) under a nitrogen atomosphere was added $Pd(PPh_3)_4$ (49 mg, 42 µmol, 0.5 eq). The reaction was heated to 85 °C left to stir for 3 h before further $Pd(PPh_3)_4$ (49 mg, 42 µmol, 0.5 eq) was added. A further 30 min of stirring at 85 °C saw consumption of starting material. The reaction was therefore adjudged complete and cooled to RT and concentrated by the azeotropic removal of AcOH with toluene. The resulting residue was purified by silica gel column chromatography, eluting with ethylacetate and petroleum ether (30:70, 70:30, 100:0), then again with ethylacetate and petroleum ether (20:80, 40:60, 50:50, 70:30) and then finally with methanol and chloroform (3:97, 4:96, 5:95). This afforded (+)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **153** (42 mg, 43% yield) as a colourless gum. The analysis of this material was in good agreement with the data given above.

Method 3

To a solution of (+)-1D-2,6-bis-*O*-benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **152** (133 mg, 113 µmol, 1 eq) in dry MeOH (10 mL) under a nitrogen atomosphere was added $PdCl_2$ (20 mg, 113 µmol, 1 eq) the reaction left to stir at RT. After 2 h, further $PdCl_2$ (20 mg, 113 µmol, 1 eq) was added and the reaction stirred for another 1 h before the Pd residues were removed by filtration through Celite®. The filtrate was concentrated under vacuum and the residue partitioned between CH_2Cl_2 (20 mL) and a solution of H_2O_2 (15% w/w, 30 mL) the layers were stirred vigorously together for 30 min. The layers were separated and the aqueous extracted with CH_2Cl_2 (3× 10 mL). The combined organic layers were washed with brine then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with methanol and chloroform (2:98) then with ethylacetate and petroleum ether (30:70, 70:30, 100:0). This afforded *(+)-1D-2,6-bis-O-benzyl-myo-inositol 1,3,4-tris(dibenzylphosphate)* **153** (84 mg, 65% yield) as a colourless gum. The analysis of this material was in good agreement with the data given above.

Benzyloxy-*N*,*N*-diisopropylamino methoxy phosphoramidite 158

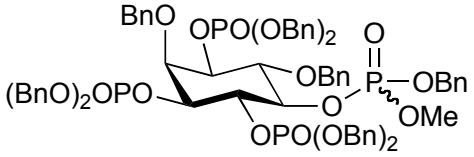


158

Benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **141** (5 g, 14.77 mmol, 1.0 eq) and 1*H*-tetrazole (0.43 M solution in acetonitrile, 13.75 mL, 5.91 mmol, 0.4 eq) were dissolved in dry CH_2Cl_2 (50 mL) under a nitrogen atmosphere. A solution of methanol (473 mg, 597 µL, 14.77 mmol, 1 eq) in dry CH_2Cl_2 (25 mL) was added dropwise *via* a dropping funnel at RT over 1 h. The reaction was stirred at RT and TLC analysis indicated the reaction had gone to completion after 3 h. The reaction was concentrated under vacuum and the residue purified by silica gel

column chromatography, eluting with triethyl amine, ethyl acetate and petroleum ether (5:5:90). This gave phophoramidite **158** (3 g, 76% yield) as a colourless oil: δ_H (300 MHz, $CDCl_3$) 7.45-7.25 (5H, m, Ar*H*), 4.70 (1H, dd, J_{AB} 12.5, J_{HP} 8.1, OCH_AH$_B$-Ph), 4.71 (1H, dd, J_{AB} 12.5, J_{HP} 8.4, OCH$_A$$H_B$-Ph), 3.76-3.61 (2H, m, 2 × -N(C*H*(CH_3)$_2$), 3.47 (3H, d, J_{HP} 13.2, -OCH_3), 1.24 (6H, d, *J* 6.8, -NCH(CH_3)$_2$), 1.23 (6H, d, *J* 6.8, -NC'H(CH_3)$_2$), δ_p (121 MHz; $CDCl_3$) 149.0. These data are in good agreement with the literature values.[128]

(-)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-methyl)phosphate-1,3,4-tris(dibenzylphosphate) 156



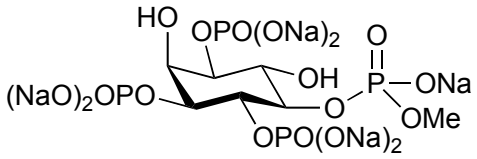
(-)-156

To a solution of alcohol **(+)-153** (100 mg, 87.6 µmol, 1 eq) in dry CH_2Cl_2 (10 mL) under a nitrogen atomosphere was added 1*H*-tetrazole (0.43 M solution in acetonitrile, 389 µL, 175.3 µmol, 2 eq) followed by phosphoramidite **158** (47 mg, 175.3 µmol, 2 eq) the reaction left to stir at RT for 2 h. After this time, further 1*H*-tetrazole (0.43 M solution in acetonitrile, 389 µL, 175.3 µmol, 2 eq) and phosphoramidite **158** (47 mg, 175.3 µmol, 2 eq) were added and the reaction stirred at RT for a further 1 h. Complete consumption of the alcohol was seen by TLC analysis. The reaction was cooled to -78 °C and 3-chloroperoxybenzoic acid (70% w/w, 87 mg, 350.0 µmol, 4 eq) added before the solution was warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 10 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue

purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (50:50, 60:40, 70:30, 80:20, 90:10, 100:0) to give *(-)-1D-2,6-bis-O-benzyl-myo-inositol 5-(O-benzyl-O-methyl)-phosphate-1,3,4-tris(dibenzylphosphate)* ***156*** (91 mg, 79% yield) as a colourless gum: R_f 0.45 (4% MeOH in $CHCl_3$); $[\alpha]_D^{25}$ = -1.02 (*c* 0.98 in $CHCl_3$); υ_{max} (*NaCl disc*)/cm^{-1} 3483 (w), 3089 (m), 3064 (s), 3033 (s), 2954 (s), 2894 (m), 2732 (w), 2507 (w), 1956 (w), 1887 (w), 1814 (w), 1734 (w), 1607 (s), 1587 (s); δ_H (500 MHz, $CDCl_3$) 7.46-7.34 (2H, m, Ar*H*), 7.33-7.00 (43H, m, Ar*H*), 5.15-4.58 (20H, m, 6 × OCH_2-Ph and 2 × inositol ring, H-4 and H-2), 4.47 (1H, ddd, *J* 9.4, 9.4, $^3J_{HP}$ 9.4, inositol ring, H-5, minor diastereomer), 4.44 (1H, ddd, *J* 9.4, 9.4, $^3J_{HP}$ 9.4, inositol ring, H-5, major diastereomer), 4.37-4.30 (1H, m, inositol ring, H-1), 4.29-4.21 (1H, m, inositol ring, H-3), 4.08 (1H, dd, *J* 9.4, 9.4, inositol ring, H-6, major diastereomer), 4.06 (1H, dd, *J* 9.4, 9.4, inositol ring, H-6, minor diastereomer), 3.55 (3H, d, $^3J_{HP}$ 11.4, POCH_3, minor diastereomer), 3.33 (3H, d, $^3J_{HP}$ 11.4, POCH_3, major diasteriomer); δ_C (125 MHz; $CDCl_3$), 138.1 (Ar*C*), 138.0 (Ar*C*), 136.1 (d, $^3J_{CP}$ 7.1, Ar*C*), 136.0 (d, $^3J_{CP}$ 6.7, Ar*C*), 136.0 (d, $^3J_{CP}$ 7.7, Ar*C*), 136.0 (d, $^3J_{CP}$ 7.6, Ar*C*), 135.9 (d, $^3J_{CP}$ 7.7, Ar*C*), 135.8 (d, $^3J_{CP}$ 6.6, Ar*C*), 135.6-135.4 (m, Ar*C*), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 128.3 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.1 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.8 (Ar*C*H), 127.5 (Ar*C*H), 127.4 (Ar*C*H), 127.4 (Ar*C*H), 127.3 (Ar*C*H), 127.2 (Ar*C*H), 127.2 (Ar*C*H), 127.1 (Ar*C*H), 78.1-77.8 (m, inositol ring, C-5), 77.8-77.6 (m, inositol ring, C-6), 77.2* (inositol ring, C-2), 77.1* (inositol ring, C-1), 76.2-75.9 (m, inositol ring, C-4), 75.9 (2 × O*C*H_2-Ph, major and minor diastereomers), 75.5-75.3 (m, inositol ring, C-3), 74.8 (O*C*H_2-Ph, major diastereomer), 74.5 (O*C*H_2-Ph, minor diastereomer), 69.8-69.7 (m, ^{31}P coupled PO*C*H_2-Ph), 69.7-69.5 (m, ^{31}P coupled PO*C*H_2-Ph), 69.4-69.2 (m, ^{31}P coupled PO*C*H_2-Ph), 54.7 (d, $^2J_{CP}$ 6.5, PO*C*H_3, minor diastereomer), 54.2 (d, $^2J_{CP}$ 5.2, PO*C*H_3, major diastereomer); δ_p (161 MHz; $CDCl_3$) 1.0 (minor diastereomer), 0.9 (major diastereomer), -0.0 (major diastereomer), -0.1 (minor diastereomer), -0.4 (minor diastereomer), -0.5 (major diastereomer), -0.8 (minor and major

diastereomers); *m/z* (ES^+) [Found: $(M+Na)^+$ 1347.36 (100.0%), 1348.36 (74.3%), 1349.36 (27.4%), 1350.37 (3.8%), 1351.36 (0.6%). $C_{70}H_{72}NaO_{18}P_4$ calculated M^+, 1347.36 (100.0%), 1348.36 (77.2%), 1349.36 (33.1%), 1350.37 (10.2%), 1351.37 (2.5%)]; *m/z* (ES^+) 1426 ($[M +HNEt_3]^+$, 100%), 1347 ($[M+Na]^+$, 50%); Anal. Calcd. For $C_{70}H_{72}O_{18}P_4$: C, 63.4, H, 5.5; Found: C, 63.5, H, 5.4. *Signals were assigned from the HSQC NMR spectrum, as the solvent peak swamped the signal in the 1D ^{13}C spectrum.

(-)-1D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-methylphosphate ester 157

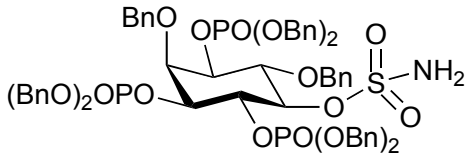


(-)-157

To a stirred solution of (-)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-methyl)phosphate-1,3,4-tris(dibenzylphosphate) **156** (44 mg, 33 µmol, 1 eq) in ^{t}BuOH (3 mL) and H_2O (0.5 mL) was added Pd(black) (64 mg, 598 µmol, 16 eq) and $NaHCO_3$ (19 mg, 232 µmol, 7 eq). The mixture was stirred at 1 atm H_2 and room temperature for 5 h. The Pd was collected *via* filtration through Celite©, and washed with Et_2O and the filtrate was collected. The Et_2O was removed under vacuum and the remaining ^{t}BuOH/H_2O freeze dried. The Pd residues were then washed with excess H_2O. The Pd washings were freeze-dried to give *(-)-1D-myo-inositol-1,3,4-trisphosphate-5-O-methylphosphate ester* **157** (27 mg, 83%) as a colourless solid: $[\alpha]_D^{25}$= -1.53 (*c* 0.26 in H_2O); υ_{max} (*KBr disc*)/cm^{-1} 3417 (s, OH), 2360 (s), 1669 (s); δ_H (500 MHz, D_2O) 4.35 (1H, dd, *J* 2.2, 2.2, inositol ring, H-2), 4.31 (1H, ddd, *J* 9.6, 9.6, $^3J_{HP}$ 9.6, inositol ring, H-4) 4.01-3.79 (4H, m, inositol ring, H-1, H-3, H-5, H-6), 3.58 (1H, d, $^3J_{HP}$ 10.9, POCH_3); δ_C (125 MHz; D_2O) 79.2 (dd, $^3J_{CP}$ 5.7, $^2J_{CP}$ 5.7, inositol ring, C-5), 75.4-74.9 (m, inositol ring, C-4), 73.7 (d, $^2J_{CP}$ 4.6, inositol ring, C-3), 73.5 (d, $^2J_{CP}$ 5.0, inositol ring, C-

1), 71.7 (d, $^{3}J_{CP}$ 4.9, inositol ring, C-6), 70.8 (inositol ring, C-2), 53.4 (d, $^{2}J_{CP}$ 5.6, PO*C*H$_3$); δ_P (101 MHz; D_2O) 5.01, 4.55, 2.88, 2.46; HRMS *m/z* (ES^-) [Found: $(M-4Na+3H)^-$ 578.8833, $(M-3Na+2H)^-$ 600.8651, $(M-2Na+H)^-$ 622.8469. $C_7H_{12}Na_5O_{18}P_4$ requires M^-, 622.8469].

(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 5-*O*-sulfamoly-1,3,4-tris(dibenzylphosphate) 159

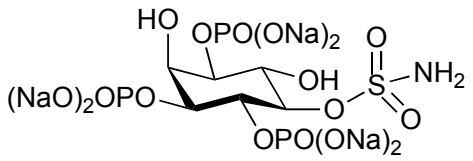


(+)-159

Alcohol **153** (88 mg, 77.1 µmol, 1 eq) was dissolved in dry DMA (1 mL) and placed under a nitrogen atomosphere and cooled to 0 °C. To this solution of alcohol was added sulfamoly chloride (2.50 M solution in CH_2Cl_2, 62 µL, 154 µmol, 2 eq) and the reaction was left to warm to RT and stir overnight. After this time, the reaction was adjudged incomplete by TLC analysis. Therefore, the reaction was cooled to 0 °C and sulfamoly chloride (2.50 M solution in CH_2Cl_2, 31 µL, 77 µmol, 1 eq) was added before the reaction was warmed to RT and stirred for 1 h. The reaction was poured onto brine (5 mL) at 0 °C and the aqueous layer extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with fresh brine and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified twice by silica gel column chromatography first eluting with MeOH and $CHCl_3$ (4:96) and then second eluting with ethyl acetate and petroleum ether (70:30, 80:20, 90:10) to give *(+)-1D-2,6-bis-O-benzyl-myo-inositol 5-O-sulfamoly-1,3,4-tris-(dibenzylphosphate)* **159** (91 mg, 50% yield) as a colourless gum: R_f 0.25 (4% MeOH in $CHCl_3$); $[\alpha]_D^{25}$ = +2.60 (*c* 0.50 in $CHCl_3$); υ_{max} (*NaCl plate*)/cm^{-1} 3384 (m), 3196 (m), 3065 (s), 3034 (s), 2960 (m), 1957 (w), 1886 (w), 1814 (w), 1740 (w),

1587 (s), 1498 (s); δ_H (400 MHz, $CDCl_3$) 7.47-7.43 (2H, m, Ar*H*), 7.33-7.20 (28H, m, Ar*H*), 7.20-7.12 (10H, m, Ar*H*), 5.92 (2H, S, NH_2), 5.08-4.96 (5H, m, POC$H_A$$H_B$-Ph), 4.98* (1H, m, OC$H_A$$H_B$-Ph), 4.96-4.78 (9H, m, 4 × POC$H_A$$H_B$-Ph + 1 × inositol ring, H-4), 4.75 (1H, d, *J* 11.4, OC$H_{A'}$$H_{B'}$-Ph), 4.70 (1H, d, *J* 11.4, OC$H_{A'}$$H_{B'}$-Ph), 4.65-4.60 (2H, m, 1 × OC$H_A$$H_B$-Ph*, 1 × inositol ring, H-2), 4.56 (1H, dd, *J* 9.6, 9.6, inositol ring, H-5), 4.17 (1H, ddd, *J* 10.1, 2.1, $^3J_{HP}$ 8.2, inositol ring, H-1), 4.13 (1H, ddd, *J* 10.0, 2.0, $^3J_{HP}$ 8.1, inositol ring, H-3), 4.03 (1H, dd, *J* 9.6, 9.6, inositol ring, H-6); δ_C (125 MHz; $CDCl_3$), 139.0 (Ar*C*), 137.4 (Ar*C*), 135.4 (d, $^3J_{CP}$ 7.6, Ar*C*), 135.4 (d, $^3J_{CP}$ 6.7, Ar*C*), 135.4 (d, $^3J_{CP}$ 6.7, Ar*C*), 135.3 (d, $^3J_{CP}$ 6.7, Ar*C*), 135.3 (d, $^3J_{CP}$ 7.6, Ar*C*), 128.6 (Ar*C*H), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.5 (Ar*C*H), 128.2 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.7 (Ar*C*H), 127.7 (Ar*C*H), 127.4 (Ar*C*H), 81.1 (inositol ring, C-5), 77.1* (inositol ring, C-2), 77.0* (inositol ring, C-6), 76.7* (inositol ring, C-1), 76.1* (inositol ring, C-4), 75.9 (O*C*H_2-Ph), 75.7 (O*C*H_2-Ph), 75.2 (dd, $^2J_{CP}$ 4.8, $^3J_{CP}$ 4.8, inositol ring, C-3), 70.2 (d, $^2J_{CP}$ 5.7, PO*C*H_2-Ph), 70.2 (d, $^2J_{CP}$ 5.7, PO*C*H_2-Ph), 69.8 (d, $^2J_{CP}$ 5.7, PO*C*H_2-Ph), 69.7 (d, $^2J_{CP}$ 5.8, PO*C*H_2-Ph), 69.6 (d, $^2J_{CP}$ 5.5, PO*C*H_2-Ph), 69.4 (d, $^2J_{CP}$ 4.9, PO*C*H_2-Ph); δ_p (161 MHz; $CDCl_3$) -0.58, -0.62, -0.67; *m/z* (ES^+) [Found: $(M+Na)^+$ 1242.29 (100.0%), 1243.29 (67.7%), 1244.29 (30.4%), 1245.29 (11.5%), 1246.30 (3.1%). $C_{70}H_{72}NaO_{18}P_4$ calculated M^+, 1242.30 (100.0%), 1243.30 (69.6%), 1244.30 (31.9%), 1245.31 (10.9%), 1246.31 (3.0%)]; *m/z* (ES^+) 1278 ($[M+NH_4 \cdot MeCN]^+$, 100%), (ES^-) 1218 ($[M-H]^-$, 100%); Anal. Calcd. For $C_{62}H_{64}NO_{17}P_3S$: C, 61.0, H, 5.3, N, 1.2, S, 2.6; Found: C, 61.1, H, 5.2, N, 1.2, S, 2.6. *Signals were assigned from the HSQC NMR spectrum.

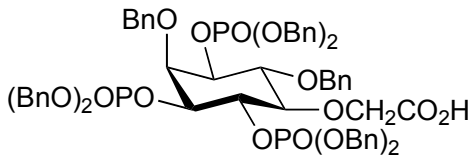
(+)-1D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-sulfamate 160



(+)-160

To a stirred solution of (+)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 5-*O*-sulfamoly-1,3,4-tris(dibenzylphosphate) **159** (45 mg, 37 µmol, 1 eq) in ^{t}BuOH (3 mL), MeOH (1 mL) and H_2O (0.5 mL) was added Pd(black) (63 mg, 590 µmol, 16 eq) and $NaHCO_3$ (18 mg, 221 µmol, 6 eq). The mixture was stirred at 1 atm H_2 and room temperature for 5 h. The Pd was collected *via* filtration through Celite®, and washed with Et_2O the filtrate was collected. The Et_2O was removed under vacuum and the remaining ^{t}BuOH/H_2O freeze-dried. The Pd residues were then washed with excess H_2O. The Pd washings were then freeze dried to give *(+)-1D-myo-inositol-1,3,4-trisphosphate-5-O-methylphosphate ester* **160** (20 mg, 84%) as a colourless solid. $[\alpha]_D^{25}$= +3.08 (*c* 0.20 in H_2O); υ_{max} (*KBr disc*)/cm^{-1} 3418 (s, OH), 1632 (m), 1357 (s) 975 (s); δ_H (500 MHz, D_2O) 4.47-4.37 (2H, m, 2 × inositol ring, H-4 and H-2), 4.32 (1H, dd, *J* 9.0, 9.0, inositol ring, H-5), 4.00-3.87 (3H, m, 3 × inositol ring, H-1, H-3 and H-6); δ_C (125 MHz; $CDCl_3$) 84.3 (inositol ring, C-5), 73.7-73.5 (m, inositol ring, C-4), 73.4 (d, $^2J_{CP}$ 3.0, inositol ring, C-3), 73.3 (d, $^2J_{CP}$ 4.9, inositol ring, C-1), 70.3 (d, $^3J_{CP}$ 3.0, inositol ring, C-2), 70.2 (d, $^3J_{CP}$ 3.3, inositol ring, C-6); δ_P (101 MHz; D_2O) 5.4, 4.2, 3.9; HRMS *m/z* (ES$^+$) [Found: (M-4Na+3H)$^-$ 541.8919, (M-3Na+2H)$^-$ 563.8740, (M-2Na+H)$^-$ 585.8559, (M-Na) 607.8376. $C_6H_{10}NNa_5O_{17}P_3S$ requires M$^-$, 607.8376].

(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate)-5-*O*-acetic acid 172

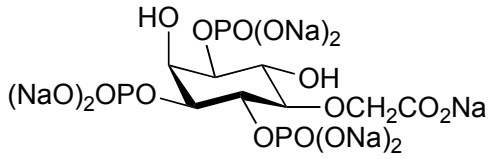


(+)-172

To a solution of (+)-1D-2,6-bis-*O*-benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) **152** (150 mg, 127 μmol, 1 eq) in a CCl_4 (3 mL), MeCN (3 mL) and H_2O (5 mL) mixture, was added $NaIO_4$ (111 mg, 521 μmol, 4.1 eq) followed $RuCl_3 \cdot H_2O$ (substoichiometric). The reaction left to stir at RT and monitored by TLC and mass spec. analysis. After 2 h TLC showed traces of starting material and mass spec. showed M-H for the product and a possible M-H for the aldehyde intermediate. Therefore further $NaIO_4$ (27 mg, 127 μmol, 1 eq) was added. After 2 h, further $NaIO_4$ (27 mg, 127 μmol, 1 eq) was added and the reaction stirred for another 2 h. The reaction was adjudged complete after this time, so was diluted with CH_2Cl_2 (15 mL) and H_2O (20 mL) the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were partitioned with the addition a solution of H_2O_2 (15% w/w, 40 mL) the layers were stirred vigorously together for 30 min. The layers were separated and the aqueous extracted with CH_2Cl_2 (2× 10 mL). The combined organic layers were washed with brine then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with ethyl acetate and acetic acid (100:0 then 99:1). This afforded (+)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate)-5-*O*-acetic acid **172** (60 mg, 40% yield) as a colourless gum: R_f 0.10 (100% ethyl acetate); $[\alpha]_D^{25}$ = +3.20 (*c* 0.97 in $CHCl_3$); υ_{max} (*NaCl plate*)/cm^{-1} 3500 (w br), 3089 (m), 3064 (s), 3034 (s), 2960 (m), 2922 (M) 1957 (w), 1886 (w), 1750 (m), 1263 (s), 1018 (s); δ_H (500 MHz, $CDCl_3$) 7.41-7.07 (40H, m, Ar*H*), 5.09-4.85 (12H, m, 6 × POCH_AH_B-Ph), 4.80 (1H, d, *J* 10.7, OCH_AH_B-Ph), 4.78-4.68 (3H, m, OC$H_{A'}H_{B'}$-Ph + inositol ring, H-4), 4.63 (1H, d, *J* 10.7,

OCH_AH_B-Ph), 4.56 (1H, dd, *J* 2.4, 2.4, inositol ring, H-2), 4.51 (1H, d, *J* 14.7, $OCH_{A''}H_{B''}$-CO_2H), 4.25-4.18 (2H, m, inositol ring, H-1 and H-3), 4.00 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.99 (1H, d, *J* 14.7, $OCH_{A''}H_{B''}$-CO_2H), 3.16 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5); δ_C (125 MHz; $CDCl_3$), 169.8 (C=O), 137.9 (Ar*C*), 136.9 (Ar*C*), 135.4 (d, $^2J_{CP}$ 6.6, Ar*C*), 135.3 (d, $^2J_{CP}$ 7.8, Ar*C*), 135.2 (d, $^2J_{CP}$ 6.8, Ar*C*), 135.0 (d, $^2J_{CP}$ 6.2, Ar*C*), 128.8 (Ar*C*H), 128.6 (Ar*C*H), 128.6 (Ar*C*H), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 128.0 (Ar*C*H), 127.9 (Ar*C*H), 127.9 (Ar*C*H), 127.7 (Ar*C*H), 127.3 (Ar*C*H), 81.2 (inositol ring, C-5), 79.2 (d, $^3J_{CP}$ 7.2, inositol ring, C-6), 77.5 (inositol ring, C-2), 77.4 (d, $^2J_{CP}$ 6.1, inositol ring, C-1), 76.5 (dd, $^2J_{CP}$ 6.5, $^3J_{CP}$ 6.5, inositol ring, C-4), 76.0 (OCH_2-Ph), 75.6 (OCH_2-Ph), 75.3 (dd, $^2J_{CP}$ 5.0, $^3J_{CP}$ 5.0, inositol ring, C-3), 71.7 (OCH_2CO_2H), 70.4 (d, $^2J_{CP}$ 5.6, $POCH_2$-Ph), 70.3 (d, $^2J_{CP}$ 5.6, $POCH_2$-Ph), 69.7 (d, $^2J_{CP}$ 5.6, $POCH_2$-Ph), 69.7 (d, $^2J_{CP}$ 5.2, $POCH_2$-Ph), 69.6 (d, $^2J_{CP}$ 5.2, $POCH_2$-Ph), 69.5 (d, $^2J_{CP}$ 5.4, $POCH_2$-Ph); δ_P (101 MHz; $CDCl_3$) 0.95, -0.20, -0.40; *m/z* (ES^+) [Found: $(M+Na)^+$ 1221.34 (100.0%), 1222.35 (70.1%), 1223.35 (26.4%), 1224.36 (4.9%). $C_{64}H_{65}O_{17}P_3Na$ calculated M^+, 1221.33 (100.0%), 1222.34 (70.6%), 1223.34 (28.1%), 1224.34 (8.1%), 1225.34 (1.9%)]; *m/z* (ES^+) 1199 ($[M+H]^+$, 100%), 1221 ($[M+Na]^+$, 95%), 1221 ($[M+HNEt_3]^+$, 40%), (ES^-) 1197 ($[M-H]^-$, 100%); Anal. Calcd. For $C_{64}H_{65}NO_{17}P_3$: C, 64.1, H, 5.5; Found: C, 64.1, H, 5.6.

(-)-1D-*myo*-Inositol 1,3,4-trisphosphate-5-*O*-acetic acid 173



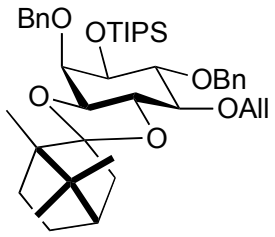
(-)-173

To a stirred solution of (+)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate)-5-*O*-acetic acid **172** (37 mg, 31 μmol, 1 eq) in ^{t}BuOH

(3 mL) and H_2O (0.5 mL) was added Pd(black) (53 mg, 501 μmol, 16 eq) and $NaHCO_3$ (18 mg, 219 μmol, 7 eq). The mixture was stirred at 1 atm H_2 and room temperature for 5 h. The Pd was collected *via* filtration through Celite®, and washed with Et_2O the filtrate was collected. The Et_2O was removed under vacuum and the remaining $^tBuOH/H_2O$ freeze-dried. The Pd residues were then washed with excess H_2O. The Pd washings were then freeze-dried to give *(-)-1D-myo-inositol 1,3,4-trisphosphate-5-O-acetic acid* **173** (17 mg, 85%) as a colourless solid. $[\alpha]_D^{25}$ = -1.76 (*c* 0.17 in H_2O); υ_{max} (*KBr disc*)/cm^{-1} 3450 (br s, OH), 1591 (s), 1455 (m), 1169 (s), 1080 (s), 993 (s); δ_H (500 MHz; D_2O) 4.39-4.21 (3H, m, 2 × inositol ring + $OCH_AH_BCO_2Na$), 4.09 (1H, d, *J* 17.4, $CH_AH_BCO_2Na$), 3.96 (1H, dd, *J* 8.5, 8.5, inositol ring), 3.92-3.76 (2H, m, 2 × inositol ring), 3.30 (1H, dd, *J* 7.9, 7.9, inositol ring); δ_C (125 MHz; $CDCl_3$) 179.1 (*C*=O), 83.2-83.0 (m, inositol ring), 76.3-75.9 (m, inositol ring), 73.9 (d, $^2J_{CP}$ 5.0, inositol ring), 73.8 (d, $^2J_{CP}$ 5.0, inositol ring), 71.3 (OCH_2CO_2Na), 71.2-70.9 (m, inositol ring), 70.8 (d, $^2J_{CP}$ 4.7, inositol ring); δ_P (101 MHz; D_2O) 4.8, 4.2, 2.5; HRMS *m/z* (ES^-) [Found: $(M-4Na+3H)^-$ 542.9073, $(M-3Na+2H)^-$ 564.8891, $(M-2Na+H)^-$ 586.8704. $C_8H_{11}Na_5O_{17}P_3$ requires M^-, 586.8703].

8.5. Synthesis of 1-O-position compounds

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol (-)-121

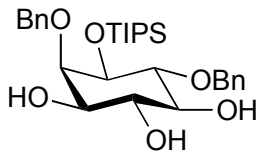


(-)-121

To a stirred solution of triisopropylsilane trifluoromethanesulfonate (2.26 g, 1.66 mL, 7.98 mmol, 4 eq) in dry CH_2Cl_2 (3 mL) under an atmosphere of nitrogen, was added triethylamine (944 mg, 1.3 mL, 9.35 mmol, 5 eq). The solution was allowed to stir for 1 h at RT, turning a dark orange colour, to this solution was added a solution of (-)-1D-5-*O*-allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-104** (3.0 g, 5.61 mmol, 1 eq) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred at RT overnight. TLC analysis indicated the reaction was complete, so it was diluted with CH_2Cl_2 (20 mL) and partitioned with the addition of water (50 mL). The layers were separated and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum to yield a dark brown oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography eluting with diethyl ether and petroleum ether (2:98) to give *(-)-1D-5-O-allyl-2,6-di-O-benzyl-1-O-triisopropylsilyl-3-O-exo-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-myo-inositol* **(-)-121** (1.12 g, 87%) as a colourless oil: R_f 0.51 (ethyl acetate and hexane 10/90); $[\alpha]_D^{25}$= -1.54 (*c* 1.23 in $CHCl_3$); υ_{max} (*NaCl plate*)/cm^{-1} 2943.5 (s), 2867.0 (s), 2362.9 (w), 1496.9 (w), 1454.2 (m), 1389.8 (m), 1369.4 (w), 1308.9 (w), 1245.9 (w), 1202.3 (w), 1181.76 (m), 1113.7 (s), 1068.2 (s),

921.6 (w), 883.4 (m), 820.3 (m), 730.4 (m), 695.5 (m), 680.7 (w); δ_H (300 MHz, $CDCl_3$) 7.38-7.12 (10H, m, Ar*H*), 5.81 (1H, dddd, *J* 17.2, 10.4, 5.5, 5.5, C*H*$_X$=CH$_Y$H$_Z$), 5.17 (1H, dd, *J* 17.2, 1.5, CH$_X$=C*H*$_Y$H$_Z$), 5.03 (1H, d, *J* 10.4, CH$_X$=CH$_Y$*H*$_Z$), 4.92 (1H, d, *J* 11.5, OC*H*$_A$H$_B$-Ph), 4.85 (1H, d, *J* 11.1, OC*H*$_{A'}$H$_{B'}$-Ph), 4.68 (1H, d, *J* 11.1, OCH$_{A'}$*H*$_{B'}$-Ph), 4.63 (1H, d, *J* 11.5, OCH$_A$*H*$_B$-Ph), 4.27 (1H, dd, *J* 12.7, 5.5, OC*H*$_V$*H*$_W$-CH=CH$_2$), 4.10 (1H, dd, *J* 2.7, 1.5, inositol ring, H-2) 4.03 (1H, dd, *J* 12.7, 5.5, OC*H*$_V$*H*$_W$-CH=CH$_2$), 3.94 (1H, dd, *J* 9.7, 9.7, inositol ring, H-6), 3.83 (1H, dd, *J* 9.0, 2.7, inositol ring, H-3), 3.67 (1H, dd, *J* 9.0, 9.0, inositol, H-4), 3.44 (1H, dd, *J* 9.7, 9.0, inositol ring, H-5), 3.21 (1H, dd, *J* 9.7, 1.5, inositol ring, H-1), 2.07 (1H, dt, *J* 13.4, 3.8, camphor ring), 1.89-1.77 (1H, m, camphor ring), 1.72-1.58 (2H, m, camphor ring), 1.43-1.36 (1H, m, camphor ring), 1.36-1.25 (1H, m, camphor ring), 1.18-1.09 (1H, m, camphor ring), 0.95-0.93 (21H, m, Si-[C*H*(C*H*$_3$)$_2$]$_3$), 0.93 (3H, s, C*H*-camphor ring), 0.77 (3H, s, C*H*$_3$-camphor bridge), 0.73 (3H, s, C*H*$_3$-camphor bridge); δ_C (75 MHz; $CDCl_3$), 139.9 (Ar*C*), 139.3 (Ar*C*), 135.8 (*C*H$_X$=CH$_Y$H$_Z$), 128.5 (Ar*C*H), 128.4 (Ar*C*H), 127.9 (Ar*C*H), 127.8 (Ar*C*H), 127.7 (Ar*C*H), 127.4 (Ar*C*H), 120.7 (ketal carbon), 116.9 (CH$_X$=*C*H$_Y$H$_Z$), 83.7 (inositol ring), 81.9 (inositol ring), 77.9 (inositol ring), 77.2 (inositol ring), 76.4 (inositol ring), 76.3 (*C*H$_2$), 75.3 (inositol ring), 74.2 (*C*H$_2$), 72.1 (*C*H$_2$), 53.3 (*C*$_q$- camphor ring), 48.7 (*C*$_q$ camphor ring), 46.7 (*C*H$_2$), 45.3 (*C*H), 29.3 (*C*H$_2$), 27.1 (*C*H$_2$), 20.7 (*C*H$_3$), 20.5 (*C*H$_3$), 18.6 (*C*H$_3$), 13.3 (*C*H$_3$), 10.0 (*C*H); *m/z* (ES$^+$) [Found: (M+Na)$^+$ 713.4209. $C_{42}H_{62}O_6SiNa$ requires *M*$^+$, 713.4213], *m/z* (ES$^+$) 713 ([M+Na]$^+$, 100%); Anal. Calcd. For $C_{42}H_{62}O_6Si$: C, 73.0, H, 9.0; Found: C, 72.7, H, 9.3.

(+)-D-2,6-bis-*O*-Benzyl-1-*O*-triisopropylsilyl-*myo*-inositol 177



(+)-177

Method 1

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-121** (458 mg, 0.66 mmol, 1 eq) was dissolved in absolute ethanol (20 mL), to this was added diisopropylethyl amine (86 mg, 115 µL, 0.66 mmol, 1 eq). The solution was stirred at room temperature for 5 minutes and then Wilkinson's catalyst (123 mg, 0.13 mmol, 0.2 eq) was added. The dark red slurry was heated under reflux for 3 h before being cooled to room temperature and filtered though Celite®. The filtrate was concentrated under vacuum and the residue analysed by 1H NMR to confirm complete isomerisation of the allylic ether to the enol ether. The crude residue was dissolved in MeOH and CH_2Cl_2 (3:2, 20 mL) and acetyl chloride (31 mg, 26 µL, 0.40 mmol, 0.6 eq) was added. The reaction was left to stir at room temperature for 4 h before the addition of triethylamine (2 mL) to quench the acid. The volatile components were removed under vacuum and the residue partitioned between ethyl acetate (20 mL) and water (20 mL) the layers separated and the aqueous phase was further extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with ethyl acetate and petroleum ether (60:40). This afforded *(+)-2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol* **(+)-177** (128 mg, 37% yield) as a colourless sticky gum: R_f 0.30 (ethyl acetate); $[\alpha]_D^{25}$ = +14.80 (*c* 1.36, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.31-7.17 (10H, m, Ar*H*), 5.01 (1H, d, *J* 11.1, OC$H_A$$H_B$-Ph), 4.88 (1H, d, *J* 11.9, OC$H_{A'}$$H_{B'}$-Ph), 4.67 (1H, d, *J* 11.1, OC$H_A$$H_B$-Ph), 4.66 (1H, d, *J* 11.9, OC$H_{A'}$$H_{B'}$-Ph), 3.83 (1H, dd, *J* 2.4, 2.4, inositol ring, H-2), 3.83 (1H, dd, *J* 9.1, 2.4, inositol

ring, H-1), 3.71 (1H, dd, *J* 9.1, 9.1, inositol ring, H-6), 3.68 (1H, ddd, *J* 9.1, 9.1, 2.4, inositol ring, H-4), 3.42 (1H, ddd, *J* 9.1, 7.8, 2.4, inositol ring, H-3), 3.30 (1H, ddd, *J* 9.1, 9.1, 2.4, inositol ring, H-5), 2.49 (1H, d, *J* 2.4, 4-O*H*), 2.30 (1H, d, *J* 2.4, 5-O*H*), 2.26 (1H, d, *J* 7.8, 3-O*H*), 1.20-1.06 (21H, m, Si-[C*H*($C*H*_3$]$_2$)$_3$); *m/z* (ES^+) 575 ([M+NH_4·MeCN]$^+$, 100%), (ES^-) 515 ([M-H]$^-$, 100%), 575 ([M+OAc]$^-$, 80%); Anal. Calcd. For $C_{29}H_{44}O_6Si$: C, 67.4, H, 8.6; Found: C, 67.3, H, 8.5. These data are in good agreement with the literature values.[31]

Method 2

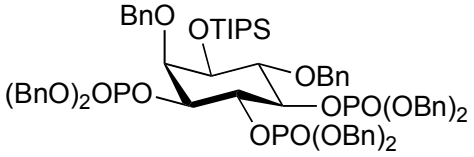
To a solution of (-)-1D-5-*O*-allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **(-)-121** (292 mg, 420 µmol, 1 eq) in AcOH (20 mL) under a nitrogen atmosphere was heated to 80 °C. $Pd(PPh_3)_4$ (244 mg, 210 µmol, 0.5 eq) was added and the reaction left to stir. After 1 h, the reaction was adjudged to be complete by TLC analysis and was cooled to RT. The AcOH was removed by azeotropic distillation with toluene *in vacuo*. The resulting black residue was purified by silica gel column chromatography, eluting with ether and hexane (50:50, 60:40, 70:30, 80:20). This afforded (+)-D-2,6-bis-*O*-benzyl-1-*O*-triisopropylsilyl-*myo*-inositol **(+)-177** (71 mg, 32% yield) as a colourless gum. The analysis of this material was in good agreement with the data given above.

Method 3

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **121** (700 mg, 1.01 mmol, 1 eq) was dissolved in MeOH (70 mL), to this was added $PdCl_2$ (179 mg, 1.01 mmol, 1 eq). The reaction was left to stir at RT for 2 h before the palladium residues were removed by filtration through Celite®. The filtrate was concentrated under vacuum and the residue partitioned between CH_2Cl_2 (50 mL) and a solution of H_2O_2 (15% w/w, 60 mL). The layers were stirred vigorously together for 30 min.

The layers were separated and the aqueous extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with sat. $NaHCO_3$ solution then brine then dried over magnesium sulfate and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with MeOH in $CHCl_3$ (1:99, 2:98 then 3:97), affording *(+)-D-2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol* **(+)-177** (426 mg, 81% yield) as a colourless gum. The analysis of this material was in good agreement with the data given above.

(+)-1D-2,6-bis-*O*-Benzyl-1-*O*-triisopropylsilyl-*myo*-inositol 3,4,5-tris(dibenzylphosphate) 178

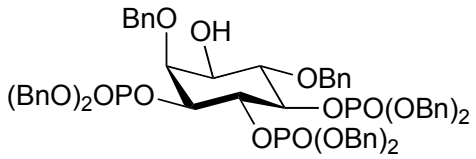


(+)-178

Bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (1.6 g, 4.64 mmol, 6 eq) was stirred under a nitrogen atomosphere with 1*H*-tetrazole (0.43M solution in acetonitrile, 10.8 mL, 4.64 mmol, 6 eq) for 20 minutes. A solution of (+)-2,6-bis-*O*-benzyl-1-*O*-triisopropylsilyl-*myo*-inositol **177** (400 mg, 0.77 mmol, 1 eq) in dry CH_2Cl_2 (40 mL) was added to the solution *via* cannular and the reaction left to stir at room temperature overnight. Further bis(benzyloxy)-*N*,*N*-diisopropylamino phosphine (535 mg, 1.55 mmol, 2 eq) and 1*H*-tetrazole (0.43M solution in acetonitrile, 3.6 mL, 1.55 mmol, 2 eq) were added and the reaction stirred at RT for 2 h. The reaction was then cooled to -78 °C and 3-chloroperoxybenzoic acid (60% w/w, 1.78 g, 6.19 mmol, 8 eq) added before being warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 40 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with a saturated

solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (40:60 then 50:50 then 60:40) to give (+)-1D-2,6-bis-*O*-benzyl-1-*O*-triisopropylsilyl-*myo*-inositol 3,4,5-tris(dibenzylphosphate) **178** (700 mg, 70% yield) as a colourless gum. R_f 0.70 (ethyl acetate/petroleum ether 60:40); $[\alpha]_D^{25}$= +4.9 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.42-7.08 (38H, m, Ar*H*), 6.85 (2H, d, *J* 7.2, Ar*H*), 5.14-4.94 (11H, m, OCH_AH_B-Ph + inositol ring, H-4), 4.92-4.81 (5H, m, OCH_AH_B-Ph), 4.78 (1H, dd, *J* 11.8, $^3J_{HP}$ 7.3, POCH_AH$_B$-Ph), 4.54 (1H, ddd, *J* 9.4, 9.4, $^3J_{HP}$ 9.4, inositol ring, H-5), 4.50 (1H, dd, *J* 11.8, $^3J_{HP}$ 8.6, POCH$_A$$H_B$-Ph), 4.38 (1H, dd, *J* 2.0, 2.0, inositol ring, H-2), 4.27 (1H, ddd, *J* 9.6, 2.0, $^3J_{HP}$ 7.2, inositol ring, H-3), 3.99 (1H, dd, *J* 9.4, 9.4, inositol ring, H-6), 3.86 (1H, dd, *J* 9.4, 2.0, inositol ring, H-1), 1.04-0.94 (21H, m, Si-[C*H*(CH_3]$_2$)$_3$); δ_p (121 MHz; $CDCl_3$) -1.5, -1.5, -1.9; *m/z* (ES^+) 1319 ($[M+Na]^+$, 30%), 1398 ($[M+HNEt_3]^+$, 50%), 1680 ($[M+Na+{}^iPr_2NPO(OBn)_2]^+$, 100%). These data are in good agreement with the literature values.[31]

(-)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 3,4,5-tris(dibenzylphosphate) 176

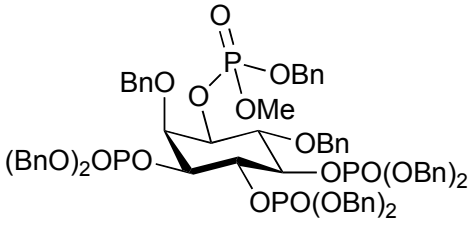


(-)-176

(+)-1D-2,6-bis-*O*-Benzyl-1-*O*-triisopropylsilyl-*myo*-inositol-3,4,5-tris-(dibenzylphosphate) **178** (1.3 g, 1.0 mmol, 1 eq) was dissolved in THF (20 mL). To this solution was added TBAF (1 M solution in THF, 1.2 mL, 1.2 mmol, 1.2 eq), the mixture was stirred at room temperature and monitored by TLC analysis. After 3 h the reaction was adjudged complete by TLC analysis and so diluted with diethyl ether (40 mL) and water (40 mL). The layers separated and

the aqueous phase further extracted with diethyl ether (3 × 20 mL). The combined organic layers were dried (magnesium sulphate), filtered and the filtrate concentrated under vacuum. The residue was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (40:60, 60:40 then 80:20). This gave (-)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 3,4,5-tris(dibenzylphosphate) **176** (912 mg, 80% yield) as a colourless gum: R_f 0.30 (petroleum ether / ethyl acetate 40:60); $[\alpha]_D^{25}$ = -7.9 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.27-7.01 (40H, m, Ar*H*), 5.06-4.83 (12H, m, 11 × POCH_AH_B-Ph + 1 × inositol ring, H-4), 4.77 (1H, dd, *J* 11.8, $^3J_{HP}$ 8.5, POCH_AH_B-Ph), 4.77 (1H, d, *J* 11.4, OCH_AH_B-Ph), 4.71 (1H, d, *J* 11.5, OC$H_{A'}H_{B'}$-Ph), 4.58 (1H, d, *J* 11.4, OCH_AH_B-Ph), 4.55 (1H, d, *J* 11.5, OC$H_{A'}H_{B'}$-Ph), 4.37 (1H, ddd, *J* 9.4, 9.4, $^3J_{HP}$ 9.4, inositol ring, H-5), 4.21 (1H, dd, *J* 2.3, 2.3, inositol ring, H-2), 4.17 (1H, ddd, *J* 9.8, 2.3, $^3J_{HP}$ 7.5, inositol ring, H-3), 3.74 (1H, dd, *J* 9.4, 9.4, inositol ring, H-6), 3.43 (1H, dd, *J* 9.4, 2.3, inositol ring, H-1); δ_p (161 MHz; $CDCl_3$) -1.2, -1.3, -1.9. These data are in good agreement with the literature values.[31]

(-)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1-*O*-benzyl-*O*-methylphosphate-3,4,5-tris(dibenzylphosphate) 199



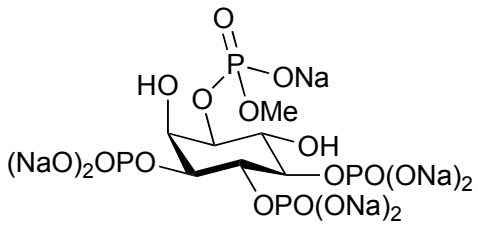
(-)-199

To a solution of alcohol **176** (50 mg, 43.8 µmol, 1 eq) in dry CH_2Cl_2 (5 mL) under a nitrogen atomosphere was added 1*H*-tetrazole (0.43 M solution in acetonitrile, 204 µL, 87.6 µmol, 2 eq) followed by aminophosphine **158** (25 mg, 87.6 µmol, 2 eq) the reaction left to stir at RT overnight. Further 1*H*-tetrazole (0.43 M

solution in acetonitrile, 204 µL, 87.6 µmol, 2 eq) followed by aminophosphine **158** (25 mg, 87.6 µmol, 2 eq) were added and the reaction stirred at RT for 2 h. The reaction was then cooled to -78 °C and 3-chloroperoxybenzoic acid (70% w/w, 43 mg, 175.3 µmol, 4 eq) added before being warmed to RT and stirred for 30 minutes. Sodium hydrogen sulfite (10% aq solution, 5 mL) was added and the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried (magnesium sulfate) and filtered. The filtrate was concentrated under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (50:50, 60:40, 70:30, 80:20) to give *(-)-1D-2,6-bis-O-benzyl-myo-inositol 1-O-benzyl-O-methylphosphate-3,4,5-tris(dibenzylphosphate)* **199** (50 mg, 86% yield) as a colourless gum. R_f 0.24 (petroleum ether / ethyl acetate 40:60); $[\alpha]_D^{25}$ = -2.13 (*c* 0.80 in $CHCl_3$); υ_{max} (*NaCl plate*)/cm^{-1} 3090 (m), 3064 (m), 3033 (m), 2957 (m), 2897 (m), 2852 (m), 1956 (w), 1886 (w), 1814 (w), 1754 (w), 1607 (s), 1587 (s), 1278 (s), 1018 (s), 738 (s), 697 (s); δ_H (500 MHz, $CDCl_3$) 7.49-7.09 (43H, m, Ar*H*), 7.04-6.95 (2H, m, Ar*H*), 5.11-4.73 (18H, m, POCH_AH_B-Ph + 2 × OCH_AH_B-Ph + inositol ring, H-4, major and minor diastereomers), 4.69-4.60 (2H, m, POCH_AH_B-Ph, inositol ring, H-2, major and minor diastereomers), 4.49 (1H, ddd, *J* 9.1, 9.1, $^3J_{HP}$ 9.1, inositol ring, H-5, major diastereomer), 4.48 (1H, ddd, *J* 9.0, 9.0, $^3J_{HP}$ 9.0, inositol ring, H-5, minor diastereomer), 4.34 (1H, ddd, *J* 9.1, 2.2, $^3J_{HP}$ 9.1, inositol ring, H-1, major diastereomer), 4.32 (1H, ddd, *J* 9.3, 2.0, $^3J_{HP}$ 9.3, inositol ring, H-1, minor diastereomer), 4.30-4.24 (1H, m, inositol ring, H-3, major and minor diastereomers), 4.09 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6, major diastereomer), 4.09 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6, minor diastereomer), 3.54 (3H, d, $^3J_{HP}$ 11.3, POCH_3, minor diastereomer), 3.49 (3H, d, $^3J_{HP}$ 11.3, POCH_3, major diastereomer); δ_C (125 MHz; $CDCl_3$), 138.1 (Ar*C*), 138.06 (Ar*C*), 136.06 (d, $^3J_{CP}$ 7.6, Ar*C*), 136.04 (d, $^3J_{CP}$ 6.8, Ar*C*), 135.9 (d, $^3J_{CP}$ 7.5, Ar*C*), 135.8 (d, $^3J_{CP}$ 7.5, Ar*C*), 135.7 (d, $^3J_{CP}$ 7.1, Ar*C*), 135.6 (d, $^3J_{CP}$ 6.6, Ar*C*), 135.5 (d, $^3J_{CP}$ 6.5, Ar*C*), 128.57 (Ar*C*H), 128.55 (Ar*C*H), 128.52 (Ar*C*H), 128.48 (Ar*C*H), 128.3 (Ar*C*H), 128.28 (Ar*C*H), 128.26 (Ar*C*H), 128.16 (Ar*C*H), 128.14 (Ar*C*H),

128.11 (Ar*C*H), 128.05 (Ar*C*H), 128.03 (Ar*C*H), 127.98 (Ar*C*H), 127.92 (Ar*C*H), 127.87 (Ar*C*H), 127.85 (Ar*C*H), 127.80 (Ar*C*H), 127.6 (Ar*C*H), 127.5 (Ar*C*H), 127.4 (Ar*C*H), 127.3 (Ar*C*H), 127.24 (Ar*C*H), 127.20 (Ar*C*H), 127.18 (Ar*C*H), 78.2-77.9 (m, inositol ring, C-5), 77.8-77.5 (m, inositol ring, C-6), 77.2* (inositol ring, C-1), 77.1 (inositol ring, C-2), 76.2-75.9 (m, inositol ring, C-4), 75.84 (O*C*H_2-Ph, minor diastereomer), 75.82 (O*C*H_2-Ph, major diastereomer), 75.5-75.3 (m, inositol ring, C-3), 74.7 (O*C*H_2-Ph, major diastereomer), 74.6 (O*C*H_2-Ph, minor diastereomer), 69.8 (d, $^2J_{CP}$ 5.8, PO*C*H_2-Ph), 69.6 (d, $^2J_{CP}$ 5.2, PO*C*H_2-Ph), 69.58-69.4 (m, PO*C*H_2-Ph), 69.3-69.2 (m, PO*C*H_2-Ph), 54.42 (d, $^2J_{CP}$ 6.0, PO*C*H_3, major diastereomer), 54.37 (d, $^2J_{CP}$ 7.1, PO*C*H_3, minor diastereomer); δ_p (161 MHz; $CDCl_3$) -0.4 (minor diastereomer), -0.7 (major diastereomer), -1.3 (major diastereomer), -1.3 (minor diastereomer), -1.5 (major diastereomer), -1.5 (minor diastereomer), -2.1 (major diastereomer), -2.1 (minor diastereomer); *m/z* (ES^+) [Found: $(M+Na)^+$ 1347.36 (100.0%), 1348.36 (73.7%), 1349.36 (30.4%), 1350.37 (8.6%), 1351.36 (1.2%). $C_{70}H_{72}NaO_{18}P_4$ calculated M^+, 1347.36 (100.0%), 1348.36 (77.2%), 1349.36 (33.1%), 1350.37 (10.2%), 1351.37 (2.5%)]; *m/z* (ES^+) 1426 ($[M +HNEt_3]^+$, 100%), 1347 ($[M+Na]^+$, 40%), 1342 ($[M+NH_4]^+$, 25%); Anal. Calcd. For $C_{70}H_{72}O_{18}P_4$: C, 63.4, H, 5.5; Found: C, 63.5, H, 5.6.

(-)-1D-*myo*-Inositol 1-*O*-methylphosphate-3,4,5-tris(phosphate) 198

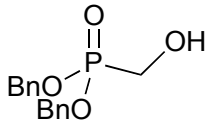


(-)-198

To a stirred solution of (-)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1-*O*-benzyl-*O*-methylphosphate-3,4,5-tris(dibenzylphosphate) **199** (45 mg, 34 μmol, 1 eq) in ^{t}BuOH (3 mL) and H_2O (0.5 mL) was added Pd(black) (65 mg, 611 μmol, 18 eq)

and $NaHCO_3$ (20 mg, 237 μmol, 7 eq). The mixture was stirred at 1 atm of H_2 and at room temperature for 5 h. The Pd was collected *via* filtration through Celite®, and washed with Et_2O the filtrate was collected. The Et_2O was removed under vacuum and the remaining $^tBuOH/H_2O$ freeze-dried. The Pd residues were then washed with excess H_2O. The Pd washings were then freeze dried to give *(-)-1D-myo-inositol 1-O-methylphosphate-3,4,5-tris(phosphate)* **198** (22 mg, 100% yield) as a colourless foam. $[\alpha]_D^{25}$= -22.36 (*c* 0.16 in H_2O); υ_{max} (*KBr disc*)/cm^{-1} 3418 (broad, s, OH), 1667 (s), 1360 (m), 1100 (s), 971 (s); δ_H (500 MHz, D_2O) 4.42 (1H, br s, inositol ring), 4.33-4.20 (1H, m, inositol ring), 4.02-3.88 (2H, m, inositol ring), 3.88-3.79 (2H, m, inositol ring), 3.55 (3H, d, $^3J_{HP}$ 10.9, POC*H*$_3$); δ_C (125 MHz; D_2O), 77.2 (inositol ring), 75.5 (d, $^2J_{CP}$ 6.2, inositol ring), 75.4-75.2 (m, inositol ring), 73.6 (inositol ring), 71.7 (d, 77.2 $^2J_{CP}$ 7.5, inositol ring), 70.2 (inositol ring), 53.0 (d, $^2J_{CP}$ 5.8, PO*C*H$_3$); δ_p (121 MHz; D_2O) 4.5, 3.4, 2.1, 1.0; HRMS *m/z* (ES^-) [Found: $(M-4Na+3H)^-$ 578.8833, $(M-3Na+2H)^-$ 600.8648, $(M-2Na+H)^-$ 622.8463. $C_7H_{12}Na_5O_{18}P_4$ requires M^-, 622.8468].

Hydroxymethyl phosphonic acid dibenzyl ester 184

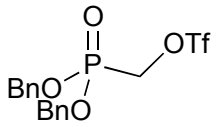


184

Paraformaldehyde (572 mg, 19.06 mmol, 1 eq) and Et_3N (193 mg, 265 μL, 1.91 mmol, 0.1 eq) were added with stirring to dibenzyl phosphite (5 g, 4.2 mL, 19.06 mmol, 1 eq). The mixture was heated to 130 °C for 20 min before being cooled to RT. The product was purified directly by silica gel column chromatography, eluting with Et_2O / EtOH (100:0 then 99:1). This afforded hydroxymethyl-phosphonic acid dibenzyl ester **184** (1.4 g, 25% yield) as a colourless oil. R_f 0.25 (Et_2O); δ_H (400 MHz, $CDCl_3$) 7.38-7.32 (10H, m, Ar*H*), 5.11

(2H, dd, J 11.9, $^3J_{HP}$ 8.8, 2 × OCH_AH$_B$-Ph), 5.04 (2H, dd, J 11.9, $^3J_{HP}$ 8.1, 2 × OCH$_A$$H_B$-Ph), 3.91 (2H, dd, J 6.8, $^2J_{HP}$ 6.1 PCH_2OH), 3.47 (1H, dt, J 6.8, $^3J_{HP}$ 4.4 OH); δ_p (162 MHz; $CDCl_3$) 25.0. These data are in good agreement with the literature values.[31]

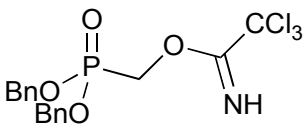
Trifluoromethanesulfonic acid bisbenzyloxyphosphorylmethyl ester 185



185

To a solution of hydroxymethylphosphonic acid dibenzyl ester **184** (800 mg, 2.74 mmol, 1 eq) in dry CH_2Cl_2 (7 mL), under a nitrogen atmosphere, and at -78 °C, was added 2,6-lutidine (352 mg, 383 µL, 3.28 mmol, 1.2 eq), followed by trifluromethane sulfonic anhydride (632 mg, 419 µL, 3.01, 1.1 eq). The reaction was stirred at -78 °C for 1 h before being warmed to -40 °C for 1 h and then at -10 °C for a further 1 h. The reaction was adjudged complete by TLC analysis and the reaction transferred *via* cannular into Et_2O (50 mL), the resulting precipitate was removed by filtration. The filtrate was washed with brine, dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure. The product was purified by silica gel column chromatography, eluting with Et_2O and hexanes (50:50). This afforded trifluoromethanesulfonic acid bisbenzyloxyphosphorylmethyl ester **185** (870 mg, 75% yield) as a colourless oil: R_f 0.56 (Et_2O); δ_H (400 MHz, $CDCl_3$) 7.44-7.31 (10H, m, ArH), 5.14 (2H, dd, J 11.6, $^3J_{HP}$ 9.6, 2 × OCH_AH$_B$-Ph), 5.09 (2H, dd, J 11.6, $^3J_{HP}$ 9.6, 2 × OCH_AH$_B$-Ph), 4.47 (2H, d, $^2J_{HP}$ 8.8, PCH_2OSO$_2$CF$_3$); δ_P (162 MHz; $CDCl_3$) 13.4; δ_F (377 MHz; $CDCl_3$) -73.8. These data are in good agreement with the literature values.[31]

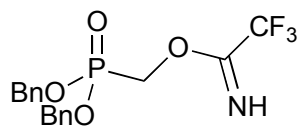
Trichloroacetimidate bisbenzyloxyphosphorylmethyl ester 187



187

To a solution of hydroxymethylphosphonic acid dibenzyl ester **184** (1.0 g, 3.42 mmol, 1 eq) and trichloroacetonitrile (2.5 g, 1.7 mL, 17.11 mmol, 5 eq) in dry CH_2Cl_2 (35 mL) under nitrogen at 0 °C, was added DBU (208 mg, 205 µL, 1.37 mmol, 0.4 eq). The reaction was allowed to warm to RT and adjudged complete by TLC analysis after 30 min. The reaction was concentrated under reduced pressure and the resulting oily residue was purified directly by silica gel column chromatography, eluting with Et_2O (100%). This afforded trichloroacetimidate bisbenzyloxyphosphorylmethyl ester **187** (865 mg, 58% yield) as a colourless oil. R_f 0.63 (Et_2O); δ_H (400 MHz, $CDCl_3$) 8.52 (1H, br s, N*H*), 7.40-7.31 (10H, m, Ar*H*), 5.17 (2H, dd, *J* 11.8, $^3J_{HP}$ 8.4, 2 × OCH_AH$_B$-Ph), 5.13 (2H, dd, *J* 11.8, $^3J_{HP}$ 8.3, 2 × OCH_AH$_B$-Ph), 4.62 (2H, d, $^2J_{HP}$ 8.2, PCH_2-TCA); δ_C (125 MHz; $CDCl_3$) 128.61 (Ar*C*H), 128.58 (Ar*C*H), 127.9 (Ar*C*H), 68.3 (d, $^2J_{CP}$ 6.3, PO*C*H$_2$-Ph), 61.8 (d, $^1J_{CP}$ 168.6, P*C*H$_2$-TCA); δ_P (162 MHz; $CDCl_3$) 18.6; *m/z* (ES^+) 494 ([M + NH_4·MeCN]$^+$, 100%).

Trifluoroacetimidate bis-benzyloxy-phosphorylmethyl ester 190

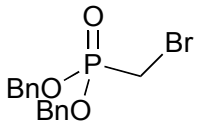


190

Trifluoroacetamide (618 mg, 5.47 mmol, 3.2 eq) was dissolved into dry CH_2Cl_2 (12 mL) under nitrogen and to this solution was added DMSO (1.28 g, 1.2 mL,

16.42 mmol, 9.6 eq). The mixture was cooled to -78 °C and freshly distilled oxalyl chloride (651 mg, 434 µL, 5.13 mmol, 3 eq) followed by dry Et_3N (1.31 g, 1.7 mL, 13.00 mmol, 7.6 eq) were added (Note: That effervescence was observed upon the addition of the oxalyl chloride). The reaction mixture was stirred at -78 °C for 40 min before the addition of DBU (521 mg, 511 µL, 3.42 mmol, 2 eq) and hydroxymethylphosphonic acid dibenzyl ester **184** (500 mg, 1.71 mmol, 1 eq). The reaction mixture was stirred at RT overnight and was adjudged complete by TLC analysis after this time. The reaction was quenched by the addition of H_2O (10 mL) and the resulting layers separated, the aqueous layer was extracted with with CH_2Cl_2 (3 × 10 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and the filtrate concentrated under reduced pressure. The product was purified by silica gel column chromatography, eluting with Et_2O (100%). This afforded trifluoroacetimidate bisbenzyloxyphosphorylmethyl ester **190** (261 mg, 40% yield) as a colourless oil. R_f 0.78 (Et_2O) δ_H (400 MHz, $CDCl_3$) 8.42 (1H, br s, N*H*), 7.42-7.31 (10H, m, Ar*H*), 5.14 (2H, dd, *J* 11.7, $^3J_{HP}$ 8.8, 2 × OC$H_A$$H_B$-Ph), 5.10 (2H, dd, *J* 11.7, $^3J_{HP}$ 8.6, 2 × OC$H_A$$H_B$-Ph), 4.54 (2H, d, $^2J_{HP}$ 8.5 PCH_2O-TFA); δ_P (162 MHz; $CDCl_3$) 18.3; δ_F (377 MHz; $CDCl_3$) -73.9; *m/z* (ES^+) 446 ([M + NH_4·MeCN]$^+$, 100%).

Bromomethylphosphonic acid dibenzyl ester 191

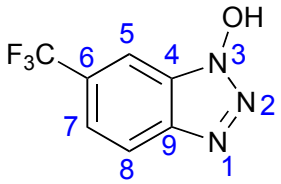


191

To a solution of triphenylphosphine (449 mg, 1.71 mmol, 1 eq) in dry Et_2O (5 mL) under nitrogen and at RT, was added CBr_4 (567 mg, 1.71 mmol, 2 eq). After 20 min of stirring at RT hydroxymethylphosphonic acid dibenzyl ester **184** (250 mg, 0.86 mmol, 1 eq) was added as a solution in Et_2O (5 mL) *via* cannular to the yellow coloured reaction mixture. The reaction was stirred overnight at RT and

after this time the now pink reaction was adjudged complete by TLC analysis. The reaction was clarified by filtration and the filtrate concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with Et_2O (100%). This afforded bromomethylphosphonic acid dibenzyl ester **191** (89 mg, 29% yield) as a colourless oil. R_f 0.47 (Et_2O); δ_H (400 MHz, $CDCl_3$) 7.41-7.31 (10H, m, Ar*H*), 5.14 (2H, dd, *J* 11.7, $^3J_{HP}$ 9.0, 2 × OCH_AH$_B$-Ph), 5.09 (2H, dd, *J* 11.7, $^3J_{HP}$ 8.6, 2 × OCH_AH$_B$-Ph), 3.23 (2H, d, $^2J_{HP}$ 9.8 PCH_2Br); δ_P (162 MHz; $CDCl_3$) 19.7; *m/z* (ES^+) 413 ([M + NH_4·MeCN]$^+$, 100%).

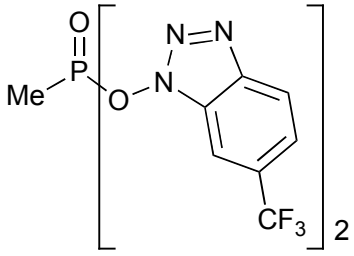
6-Trifluoromethyl-*N*-hydroxybenzotriazole 168



168

To a solution of 4-chloro-3-nitro-α,α,α-trifluorotoluene (25.0 g, 110 mmol, 1 eq) in ethanol (34 mL) was added hydrazine hydrate (16.6 g, 16.1 mL, 333 mmol, 3 eq). The reaction was heated to reflux and stirred for 18 h. The reaction was cooled to RT and ethanol removed under reduced pressure. The resulting solid residue was dissolved in an aqueous Na_2CO_3 solution (10% w/v 300 mL) and washed with Et_2O (2 × 100 mL). The aqueous solution was acidified to pH 6 by the addition of conc. HCl. The resulting solid was collected *via* filtration and washed with H_2O (200 mL), the beige crystals were dried over P_2O_5 in a vacuum dessicator. This gave 6-trifluoromethyl-*N*-hydroxybenzotriazole **168** (10.8 g, 48% yield) as a beige crystalline solid: δ_H (400 MHz, D_6-acetone) 8.19 (1H, d, *J* 8.8, *H*-7), 8.12 (1H, s, *H*-5), 7.71 (1H, d, *J* 8.8, *H*-8); *m/z* (ES^-) 202.1 ([M-H]$^-$, 80%), 405.1 ([2M-H]$^-$, 100%). These data are in good agreement with the literature values.[108]

Bis(6-trifluoromethyl-*N*-hydroxybenzotriazolyl)methylphosphonate 169

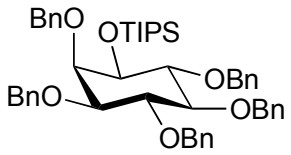


169

6-Trifluoromethyl-*N*-hydroxybenzotriazole **168** (1.53 g, 7.52 mmol, 2 eq) was dissolved in dry dioxane (20 mL) and placed under a nitrogen atmosphere. To this solution was added dry pyridine (595 mg, 608 µL, 7.52 mmol, 2 eq) followed by the dropwise addition of a solution of methylphosphonic dichloride (500 mg, 341 µL, 3.76 mmol, 1 eq) in dry dioxane (10 mL). The reaction was stirred at 20 °C for 2 h before the resulting pyridine salts were removed *via* Schlenk filtration. The filtrate was used as a 0.125 M stock solution of bis(6-trifluoromethyl-*N*-hydroxybenzotriazolyl)methylphosphonate **169**. ^{31}P NMR was recorded using a D_6-benzene capillary for internal deuterium lock. δ_P (162 MHz; D_6-benzene) 47.4. These data are in good agreement with the literature values.[108]

8.6. Synthesis of Phosphatidylinositol

(+)-D-2,3,4,5,6-Penta-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol (+)-205

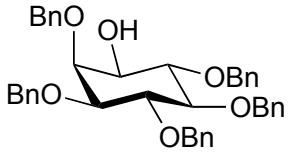


(+)-205

(-)-D-2,6-Bis-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol **(+)-177** (128 mg, 0.25 mmol, 1 eq) was dissolved in dry DMF (10 ml) under an atmosphere of nitrogen and cooled to 0°C. With vigorous stirring, sodium hydride (60% dispersion w/w in mineral oil, 40 mg, 0.99 mmol, 4 eq) was added. The resulting slurry was allowed to warm to RT and stirred for a further 2 h. The slurry was cooled to 0°C and benzyl bromide (170 mg, 117 µL, 0.99 mmol, 4 eq) was added with vigorous stirring. The reaction mixture was allowed to warm to RT and stir overnight. The reaction showed possible tris- and tetrakis- benzylated adducts by TLC analysis. Therefore the reaction was cooled to 0°C and additional sodium hydride (60% dispersion w/w in mineral oil, 40 mg, 0.99 mmol, 4 eq) and benzyl bromide (170 mg, 117 µL, 0.99 mmol, 4 eq) were added and stirred at RT for 5 h. The reaction was quenched with the addition of water (2 mL). The volatile components were removed under vacuum and the residue partitioned between ethyl acetate (20 mL) and water (20 mL). The layers were separated and the aqueous phase extracted with ethyl acetate (3 × 15 mL). The combined organic coponents were dried over magnesium sulfate, filtered and the filtrate reduced under vacuum yielding an orange oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography, eluting with ethyl acetate and hexane (1:99, 2:98 and 3:97) giving *(+)-D-2,3,4,5,6-penta-O-benzyl-1-O-triisopropylsilyl myo-inositol* ***205*** (77 mg, 68% yield) as a colourless oil: R_f 0.40 (petroleum ether / diethyl ether 90/10); $[\alpha]_D^{25}$= +1.80 (*c* 1.00 in $CHCl_3$); υ_{max} (*NaCl plate*)/cm^{-1} 3089 (w), 3064 (w), 3030 (m), 2925 (s), 2866 (s), 2361 (m), 2342 (m),

1947 (w), 1869 (w), 1808 (w), 1734 (w); δ_H (300 MHz, $CDCl_3$) 7.38-7.05 (25H, m, Ar*H*), 4.91-4.57 (10H, m, 5 × OCH_AH_B-Ph), 3.99 (1H, dd, *J* 9.5, 9.5, inositol ring, H-4), 3.87 (1H, dd, *J* 9.5, 9.5, inositol ring, H-6), 3.83 (1H, dd, *J* 2.0, 2.0, inositol ring, H-2), 3.64 (1H, dd, *J* 9.5, 2.0, inositol ring, H-1), 3.38 (1H, dd, *J* 9.5, 9.5, inositol ring, H-5), 3.34 (1H, dd, *J* 9.5, 2.0, inositol ring, H-3), 0.99-0.93 (21H, m, Si-[C*H*(CH_3)$_2$]$_3$); δ_C (100 MHz; $CDCl_3$), 139.4 (Ar*C*), 139.3 (Ar*C*) 139.0 (Ar*C*), 138.7 (Ar*C*), 138.4 (Ar*C*), 128.4 (Ar*C*H), 128.3 (Ar*C*H), 128.2 (Ar*C*H), 128.1 (Ar*C*H), 128.0 (Ar*C*H), 129.97 (Ar*C*H), 127.8 (Ar*C*H), 127.79 (Ar*C*H), 127.70 (Ar*C*H), 127.5 (Ar*C*H), 127.4 (Ar*C*H), 127.3 (Ar*C*H), 127.28 (Ar*C*H), 127.19 (Ar*C*H), 127.10 (Ar*C*H), 126.9 (Ar*C*H), 84.2 (inositol ring), 82.1 (inositol ring), 81.9 (inositol ring), 80.9 (inositol ring), 79.9 (inositol ring), 75.9 (*C*H_2), 75.7 (*C*H_2), 75.4 (*C*H_2), 74.6 (*C*H_2), 74.0 (inositol ring), 72.9 (*C*H_2), 18.21 (Si-[*C*H(CH_3)$_2$]$_3$), 18.18 (Si-[*C*H(CH_3)$_2$]$_3$), 12.8 (Si-[CH(*C*H_3)$_2$]$_3$); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 809.4204. $C_{50}H_{62}O_6SiNa$ requires M^+, 809.4208]; *m/z* (ES^+) 804 ($[M+NH_4]^+$, 45%), 809 ($[M+Na]^+$, 50%), 845 ($[M+NH_4{\cdot}MeCN]^+$, 100%); Anal. Calcd. For $C_{50}H_{62}O_6Si$: C, 76.3, H, 7.9; Found: C, 76.4, H, 7.9.

(-)-D-2,3,4,5,6-penta-*O*-benzyl *myo*-inositol (-)-201

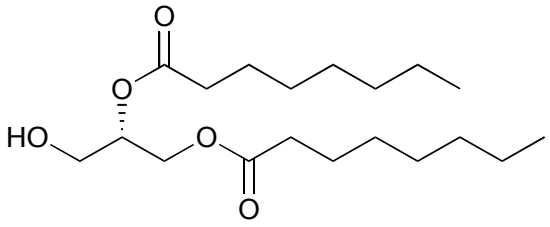


(-)-201

(-)-D-2,3,4,5,6-penta-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol **(+)-205** (136 mg, 0.17 mmol, 1 eq) was dissolved in THF (15 mL). To this solution was added TBAF (1 M solution in THF, 190 µL, 0.19 mmol, 1.1 eq), the mixture was stirred at room temperature and monitored by TLC analysis. After 3 h the reaction was adjudged complete by TLC analysis and so diluted with diethyl ether (40 mL) and water (40 mL). The layers separated and the aqueous phase further extracted

with diethyl ether (3 × 20 mL). The combined organic layers were dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum. The residue was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (20:80 then 30:70). This gave (-)-D-2,3,4,5,6-penta-*O*-benzyl *myo*-inositol **(-)-201** (96 mg, 88% yield) as a colourless gum. R_f 0.46 (petroleum ether / ethyl acetate 70:30); $[\alpha]_D^{25}$= -10.9 (*c* 0.5 in $CHCl_3$), {lit. $[\alpha]_D^{18}$= -10.0 (*c* 2.3 in $CHCl_3$)}; δ_H (300 MHz, $CDCl_3$) 7.42-7.27 (25H, m, Ar*H*), 5.06-4.70 (10H, m, 5 × OCH_AH_B-Ph), 4.09 (1H, dd, *J* 9.5, 9.5, inositol ring), 4.06 (1H, dd, *J* 2.5, 2.5, inositol ring), 3.84 (1H, dd, *J* 9.5, 9.5, inositol ring), 3.56-3.46 (3H, m, inositol ring), 2.22 (1H, d, *J* 6.3, 1-O*H*); *m/z* (ES^+) 648 ($[M+NH_4]^+$, 30%), 653 ($[M+Na]^+$, 100%), 732 ($[M+HNEt_3]^+$, 25%). These data are in good agreement with the literature values.[89,129]

1,2-Dioctanoyl-*sn*-glycerol 209

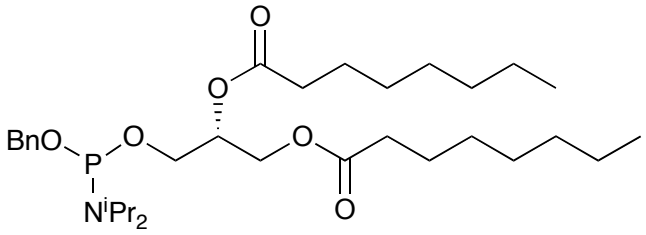


209

1,2-Dioctanoyl-3-O-benzyl-*sn*-glycerol **208** (500 mg, 1.15 mmol, 1 eq) was dissolved in dry THF (25 mL) and $Pd(OH)_2$ (50 mg, 10% w/w of benzyl ether) added. The mixture was degassed and placed under an atmosphere of hydrogen and stirred at room temperature for 36 h. The reaction was filtered through a pad of Celite® and washed with THF. The filtrate was concentrated under reduced pressure to give 1,2-dioctanoyl-*sn*-glycerol **209** (345 mg, 87% yield) as a colourless oil: δ_H (300 MHz, $CDCl_3$) 5.06-4.97 (1H, m, glycerol C*H*), 4.25 (1H, dd, *J* 12.0 , 4.6, glycerol CH_XH_Y), 4.17 (1H, dd, *J* 12.0, 5.6, glycerol

CH_XH_Y), 3.66-5.57 (1H, m, glycerol CH_2), 2.32-2.19 (4H, m, 2×αCOCH_2), 1.95 (1H, t, *J* 6.5, O*H*), 1.62-1.50 (4H, m, 2×βCOCH$_2$CH_2), 1.31-1.13 (16H, m, octanoyl CH_2), 0.81 (6H, t, *J* 6.9, 2×octanoyl CH_3). These data are in good agreement with the literature values.[130]

Benzyloxy (1,2-Dioctanoyl-*sn*-glycerol) (*N*,*N*-diisopropylamino)phosphine 203

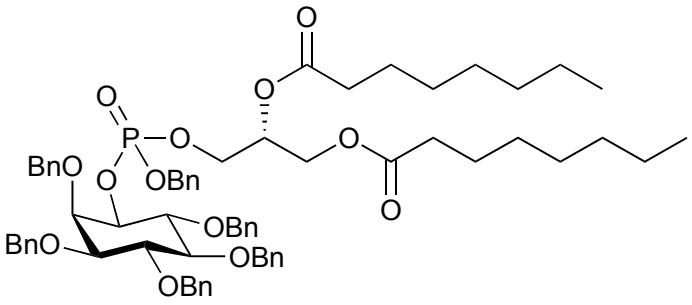


203

Benzyloxy bis(*N*,*N*-diisopropylamino)phosphine **141** (540 mg, 1.60 mmol, 1.1 eq) and 1*H*-tetrazole (0.43 M solution in acetonitrile, 1.7 mL, 725 µmol, 0.5 eq) were dissolved in dry CH_2Cl_2 (10 mL) under a nitrogen atmosphere. Alcohol **209** (500 mg, 1.45 mmol, 1 eq) as a solution dry CH_2Cl_2 (5 mL) was added dropwise, *via* cannula, over 1 h at RT. The reaction was stirred at RT and TLC analysis indicated the reaction had gone to completion after 3 h. The reaction was concentrated under reduced pressure and the crude material partitioned between CH_2Cl_2 (5 mL) and a saturated solution of $NaHCO_3$ (5 mL) the layers separated and the aqueous phase was further extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers dried over magnesium sulfate and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with triethyl amine, ethyl acetate and petroleum ether (5:15:80), gaving phophoramidite **203** (600 mg, 71% yield) as a colourless oil: δ_H (300 MHz, $CDCl_3$) 7.33-7.22 (5H, m, Ar*H*), 5.19-5.06 (1H, m, glycerol C*H*), 4.72-4.54 (2H, m, ^{i}Pr-C*H*), 4.31-4.23 (1H, m, OCH_AH_B-Ph), 4.14-4.02 (1H, m, OCH$_A$$H_B$-Ph), 3.76-4.49 (4H, m, 2×glycerol CH_2), 2.22 (4H, m, 2×αCOCH_2), 1.53

(4H, m, 2×βCOCH$_2$CH_2), 1.25-1.16 (16H, m, octanoyl CH_2), 1.15-1.08 (12H, m, 4×^{i}Pr-CH_3), 0.81 (6H, t, *J* 7.2, 2×octanoyl CH_3). δ_p (121 MHz; $CDCl_3$) 148.80, 148.68. These data are in good agreement with the literature values.[130]

(+)-D-1d-1-(1,2-Dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (+)-204

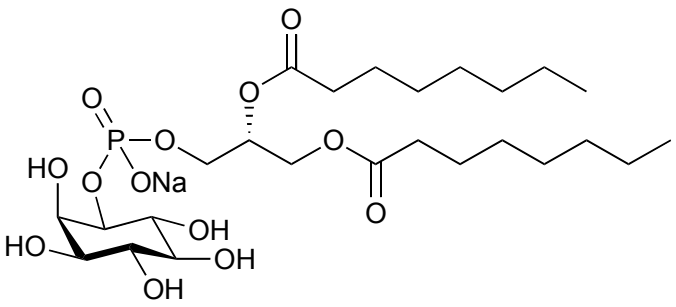


(+)-204

To a stirred solution of phosphoramidite **203** (120 mg, 0.206 mmol, 2 eq) in dry CH_2Cl_2 (5 mL) under an atmosphere of nitrogen was added 1*H*-tetrazole (0.43 M solution in acetonitrile, 479 µL, 206 µmol, 2 eq). This mixture was stirred at RT for 10 minutes before the addition of (-)-D-2,3,4,5,6-penta-*O*-benzyl *myo*-inositol **(-)-201** (65 mg, 103 µmol, 1 eq) as a solution in CH_2Cl_2 (2 mL). The reaction was stirred at RT overnight. TLC analysis confirmed that further equivalents of phosphoramidite **203** (120 mg, 0.206 mmol, 2 eq) were required and the reaction stirred at RT for another 3 h before the addition of more phosphoramidite **203** (120 mg, 0.206 mmol, 2 eq). After 2 h the reaction was cooled to -78 °C.and *m*CPBA (60% purity, 178 mg, 618 µmol, 6 eq) added. The reaction was stirred at RT for 20 min before being partitioned between sodium hydrogen sulfite (10% aq solution, 15 mL) and additional CH_2Cl_2 (5 mL), the layers separated and the aqueous layer further extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate and then dried over magnesium sulfate and filtered. The filtrate was concentrated

under vacuum and residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (20:80 then 40:60) to give (+)-D-1-(1,2-dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **(+)-204** (60 mg, 52% yield) as a colourless oil: R_f 0.66 (ethyl acetate/ petroleum ether 60:40); $[\alpha]_D^{20}$ = +3.17 (*c* 0.79 in $CHCl_3$); υ_{max} (*NaCl plate*)/cm^{-1} 3090 (s), 3064 (s), 2954 (s), 2894 (s), 1956 (w), 1885 (w), 1721 (s), 1313 (s), 1008 (s); δ_H (300 MHz, $CDCl_3$) 7.34-7.11 (30H, m, Ar*H*), 5.06-4.54 (13H, m, 6 × OCH_AH_B-Ph + glycerol C*H*), 4.28-4.24 (1H, m, inositol ring), 4.22-3.77 (7H, m, 3 × inositol ring + 2 × glycerol CH_2), 3.47-3.34 (2H, m, 2 × inositol ring), 2.26-2.06 (4H, m, 2×αCOCH_2), 1.58-1.39 (4H, m, 2 × βCOCH$_2$CH_2), 1.27-1.10 (16H, m, octanoyl CH_2), 0.85-0.75 (6H, m, 2×octanoyl CH_3); δ_C (126 MHz; $CDCl_3$), 128.6 (Ar*C*H), 128.5 (Ar*C*H), 128.34 (Ar*C*H), 128.30 (Ar*C*H), 128.27 (Ar*C*H), 128.24 (Ar*C*H), 128.19 (Ar*C*H), 128.15 (Ar*C*H), 128.0 (Ar*C*H), 127.8 (Ar*C*H), 127.77 (Ar*C*H), 127.66 (Ar*C*H), 127.60 (Ar*C*H), 127.57 (Ar*C*H), 127.53 (Ar*C*H), 127.47 (Ar*C*H), 127.45 (Ar*C*H), 127.40 (Ar*C*H), 83.1 (inositol ring), 81.2 (inositol ring), 80.4 (inositol ring), 79.9 (inositol ring), 78.6 (inositol ring), 76.3 (inositol ring), 76.0 (*C*H_2 benzylic), 75.9 (*C*H_2 benzylic), 75.5 (*C*H_2 benzylic), 75.0 (*C*H_2 benzylic), 72.7 (*C*H_2 benzylic), 69.4 (*C*H_2 benzylic), 69.0 (*C*H glycerol), 65.5 (*C*H_2 glycerol), 61.5 (*C*H_2 glycerol), 34.1 (αCO*C*H_2), 33.9 (αCO*C*H_2), 31.6 (octanoyl *C*H_2), 29.0 (octanoyl *C*H_2), 29.0 (octanoyl *C*H_2), 28.9 (octanoyl *C*H_2), 24.7 βCOCH$_2$*C*H$_2$), 22.5 (octanoyl *C*H_2), 14.0 (octanoly *C*H_3); δ_p (121 MHz; $CDCl_3$) 1.72, 1.69; *m/z* (ES^+) [Found: $(M+NH_4)^+$ 1144.5918. $C_{67}H_{87}O_{13}N_1P_1$ requires M^+, 1144.5910], *m/z* (ES^+) 1149 ($[M+Na]^+$, 100%).

(+)-Phosphatidyl inositol 200



(+)-200

To a stirred solution of (+)-D-1-(1,2-dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **(+)-204** (23 mg, 20 μmol, 1 eq) in ^{t}BuOH (8 mL) and H_2O (1.5 mL) was added Pd(black) (39 mg, 367 μmol, 18 eq) and $NaHCO_3$ (2.5 mg, 33 μmol, 1.6 eq). The mixture was stirred at 1 atm H_2 and RT for 24 h. The mixture was then filtered through Celite®, the solid washed with H_2O and the filtrate lyophilised to give phosphatidyl inositol **200** (8 mg, 64% yield) as a colourless solid: $[\alpha]_D^{25}$= +2.25 (*c* 0.20 in H_2O, pH 10), {lit. $[\alpha]_D$= +2.80 (*c* 1.0 in H_2O, pH 9)}; δ_H (500 MHz, D_2O) 5.29-5.20 (1H, m, glycerol C*H*), 4.37 (1H, d, *J* 11.7, glycerol CH_XH$_Y$-Oct), 4.24-4.12 (2H, m, glycerol CH$_X$$H_Y$-Oct + inositol ring, H-2), 4.06-3.95 (2H, m, glycerol POC$H_{X'}H_{Y'}$), 3.89 (1H, ddd, *J* 9.6, $^3J_{HP}$ 9.6, 1.9, inositol ring, H-1), 3.68 (1H, dd, *J* 9.6, 9.6, inositol ring, H-6), 3.58 (1H, dd, *J* 9.6, 9.6, inositol ring, H-4), 3.48 (1H, dd, *J* 9.6, 1.9, inositol ring, H-3), 3.27 (1H, dd, *J* 9.6, 9.6, inositol ring, H-5), 2.42-2.18 (4H, m, 2×αCOCH_2), 1.64-1.44 (4H, m, 2×βCOCH$_2$CH_2), 1.30-1.07 (16H, m, octanoyl chain, CH_2), 0.83-0.76 (6H, m, 2×octanoyl CH_3); δ_C (126 MHz; $CDCl_3$), 174.6 (*C*=O), 174.5 (*C*=O), 76.2 (d, $^2J_{CP}$ 5.6, inositol ring, C-1), 73.9 (inositol ring, C-5), 72.2 (inositol ring, C-4), 71.4 (inositol ring, C-6), 71.2 (inositol ring, C-2), 70.8-70.6 (m, glycerol PO*C*H$_2$ + inositol ring), 63.8 (d, $^2J_{CP}$ 3.8, glycerol PO*C*H$_2$), 63.2 (glycerol O*C*H$_2$-Oct), 34.1 (αCO*C*H$_2$) 34.0 (αCO*C*H$_2$), 31.8 (octanoyl chain, *C*H$_2$), 29.1 (octanoyl chain, *C*H$_2$), 29.09 (octanoyl chain, *C*H$_2$), 29.0 (octanoyl chain, *C*H$_2$), 28.9 (octanoyl chain, *C*H$_2$), 24.8 (βCOCH$_2$*C*H$_2$), 24.7 (βCOCH$_2$*C*H$_2$) 22.6 (octanoyl chain, *C*H$_2$),

22.5 (octanoyl chain, *C*H_2), 13.8 (octanoyl chain, *C*H_3), 13.7 (octanoyl chain, *C*H_3); δ_p (121 MHz; D_2O) -0.84; *m/z* (ES^-) [Found: $(M\text{-}Na)^-$ 585.2690. $C_{25}H_{46}O_{13}P_1$ requires M^-, 585.2682]; *m/z* (ES^-) 585 ($[M\text{-}Na]^-$, 100%). These data are in good agreement with the literature values.[131,132]

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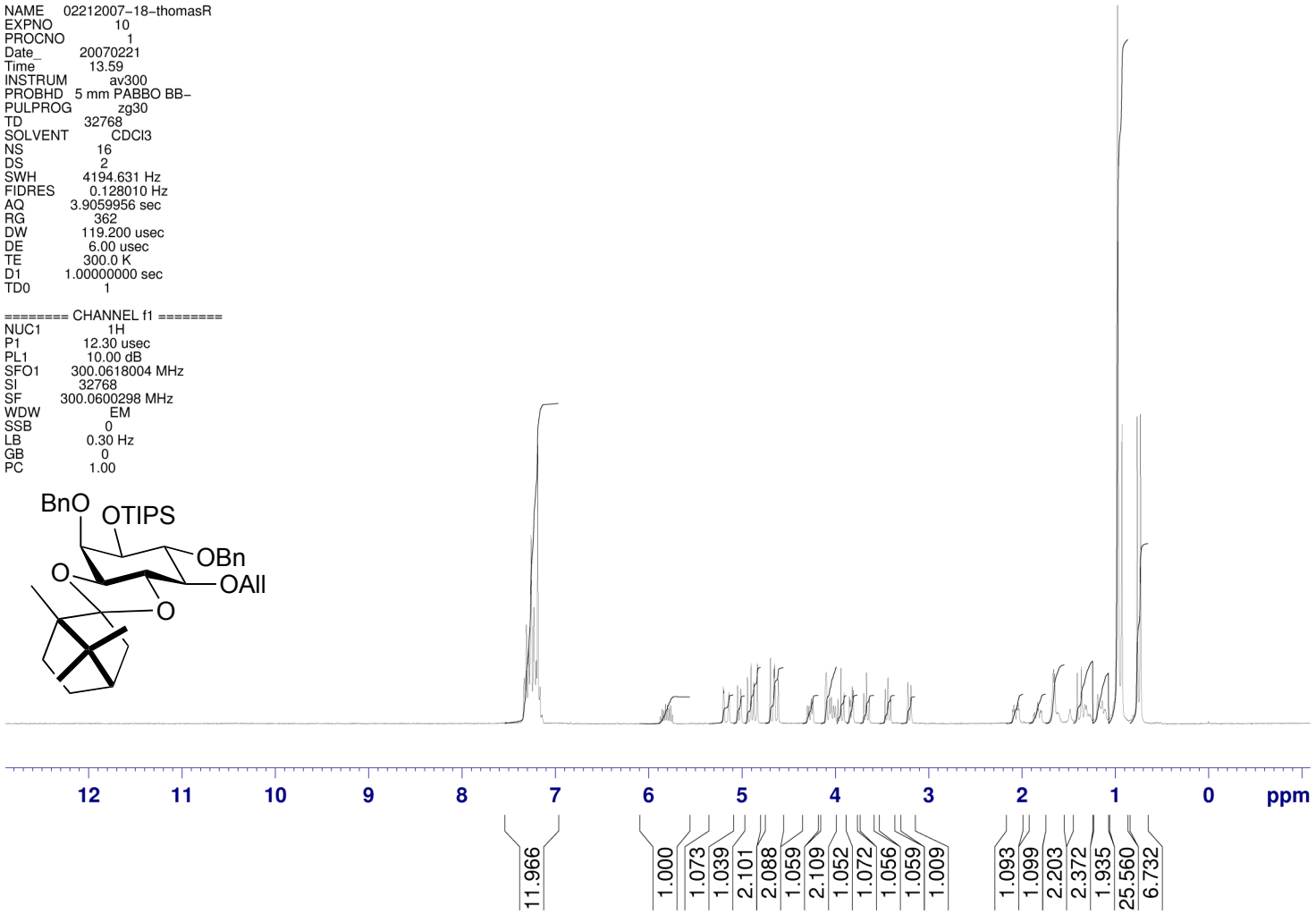
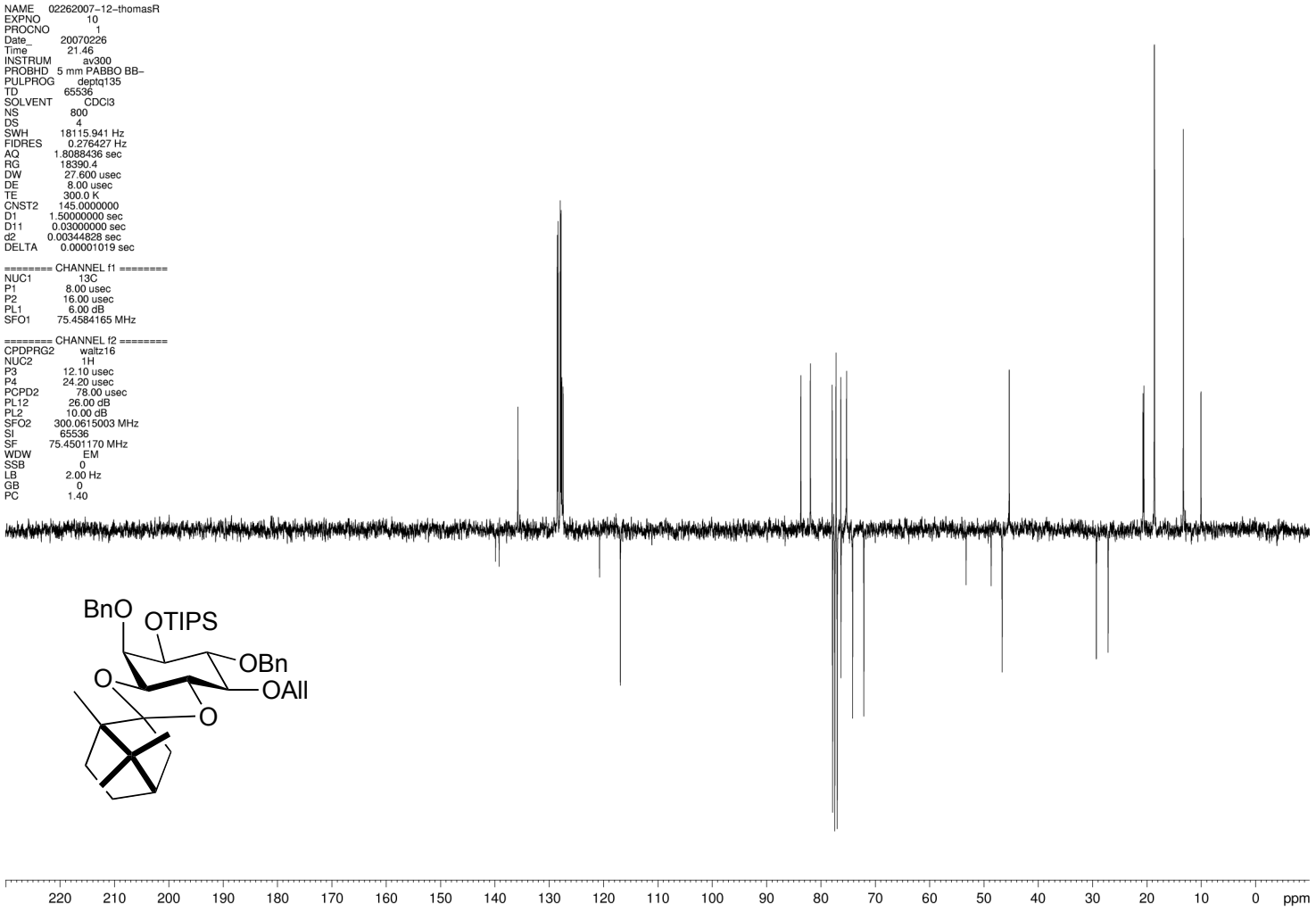
Appendix 1 – Selected NMR Spectra

(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol (-)-121



(-)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(L-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol (-)-121



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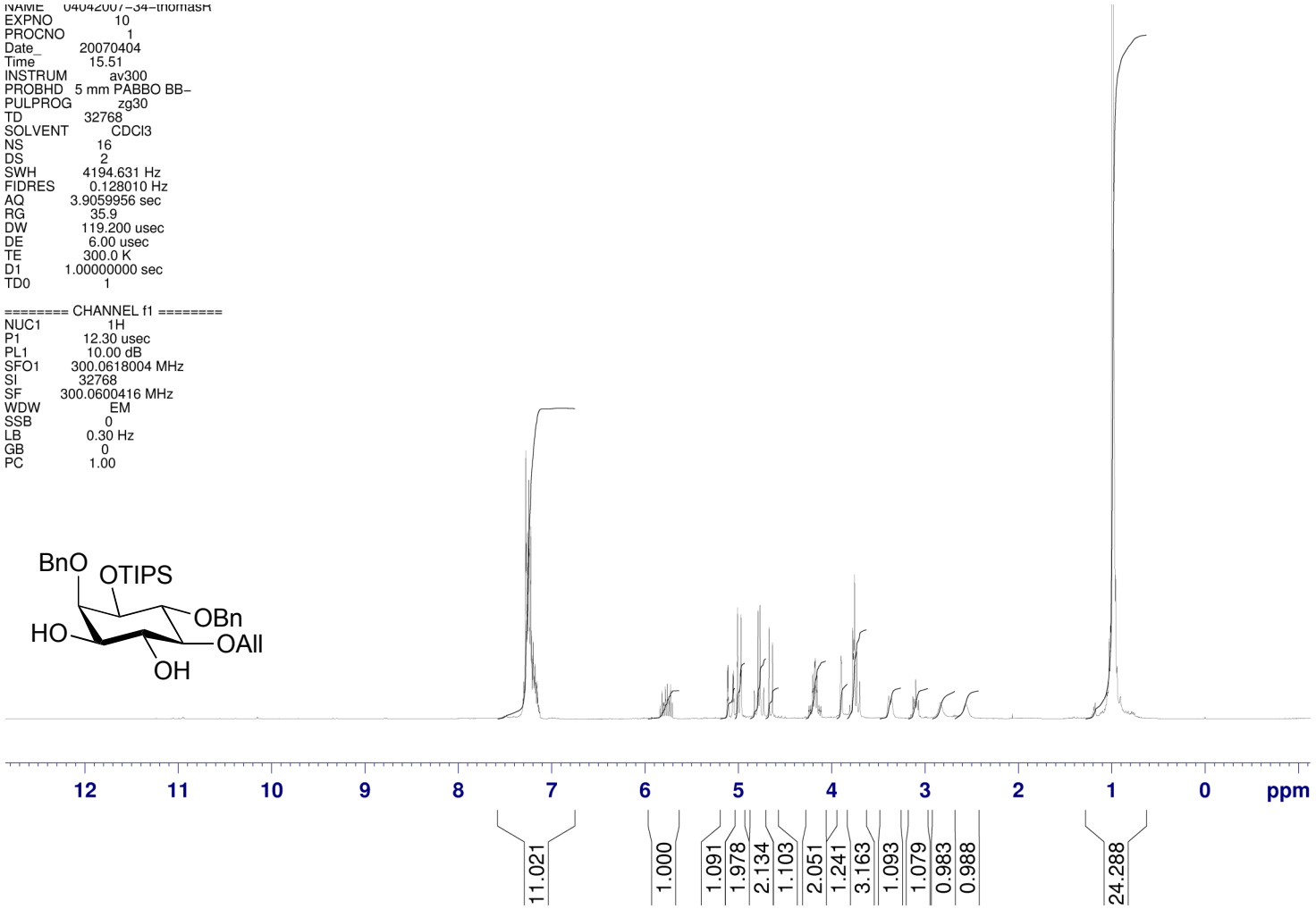

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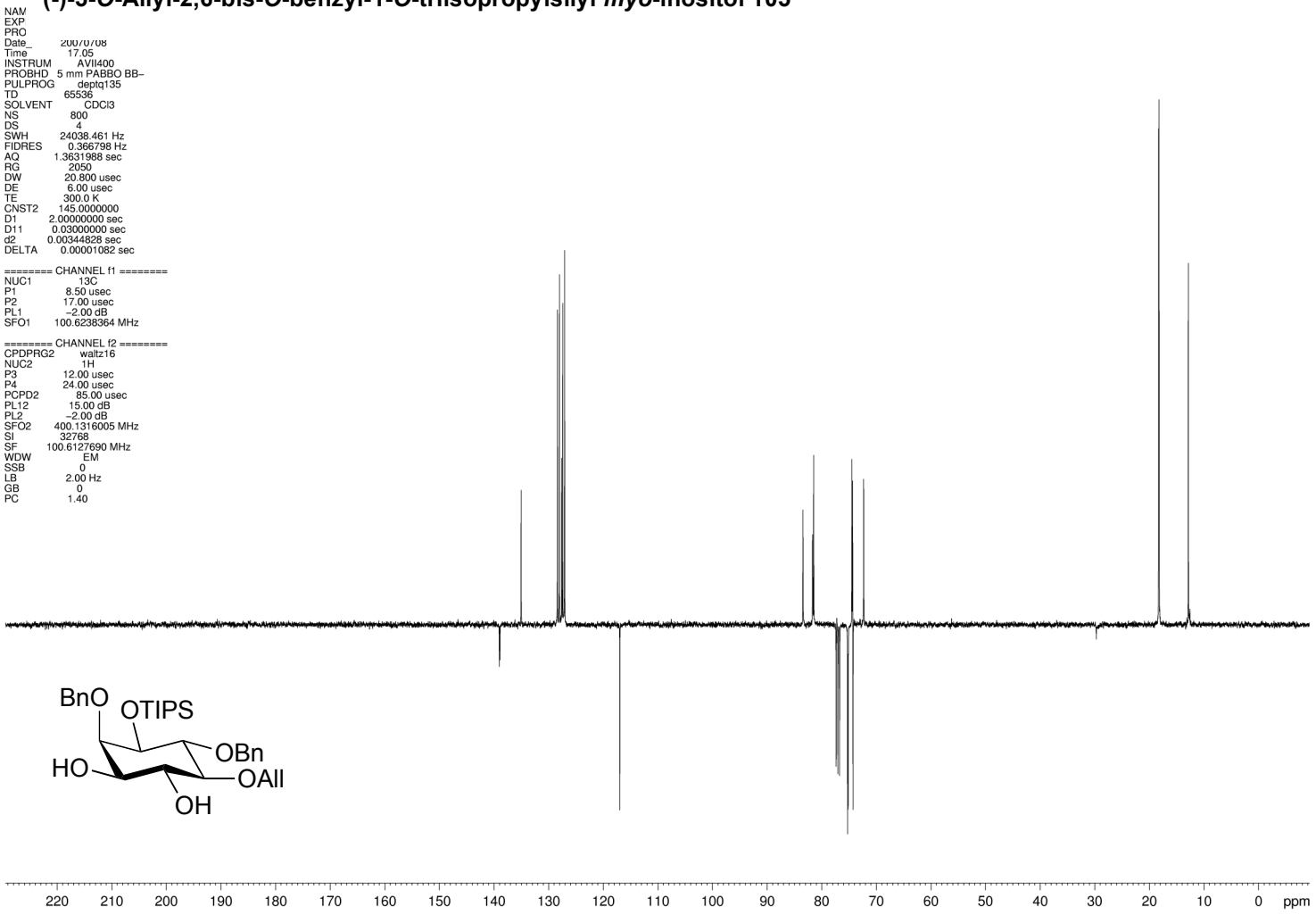

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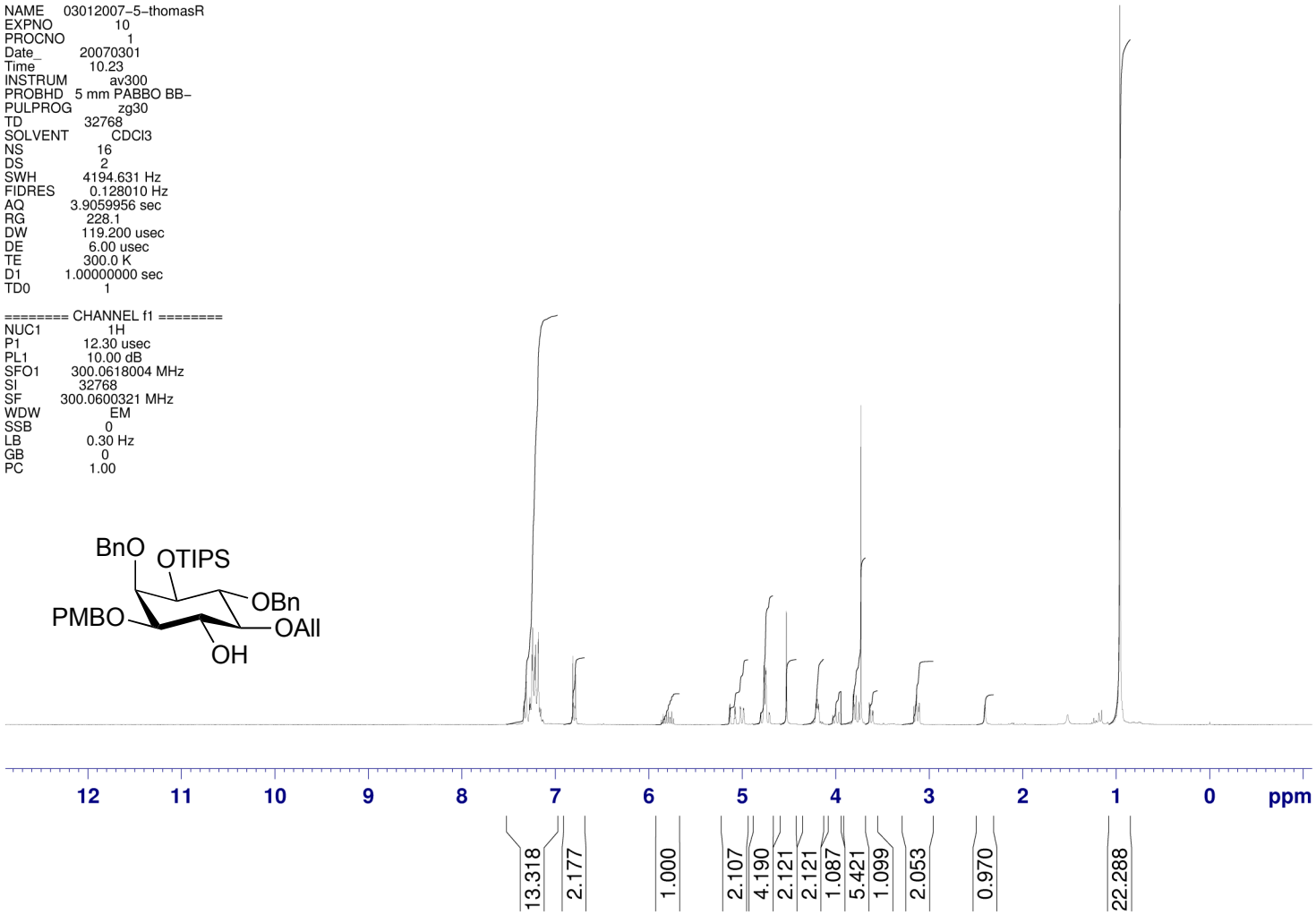

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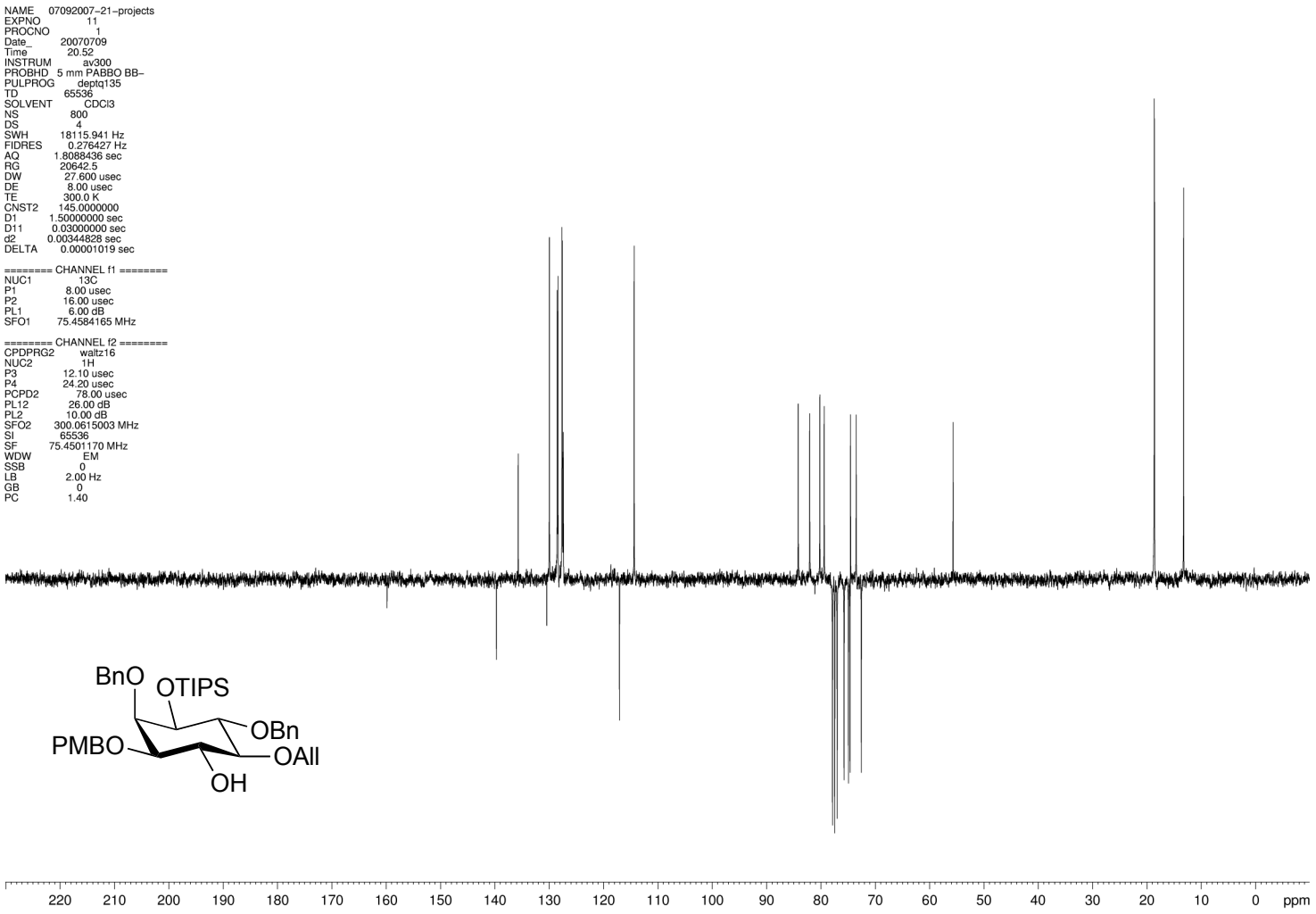

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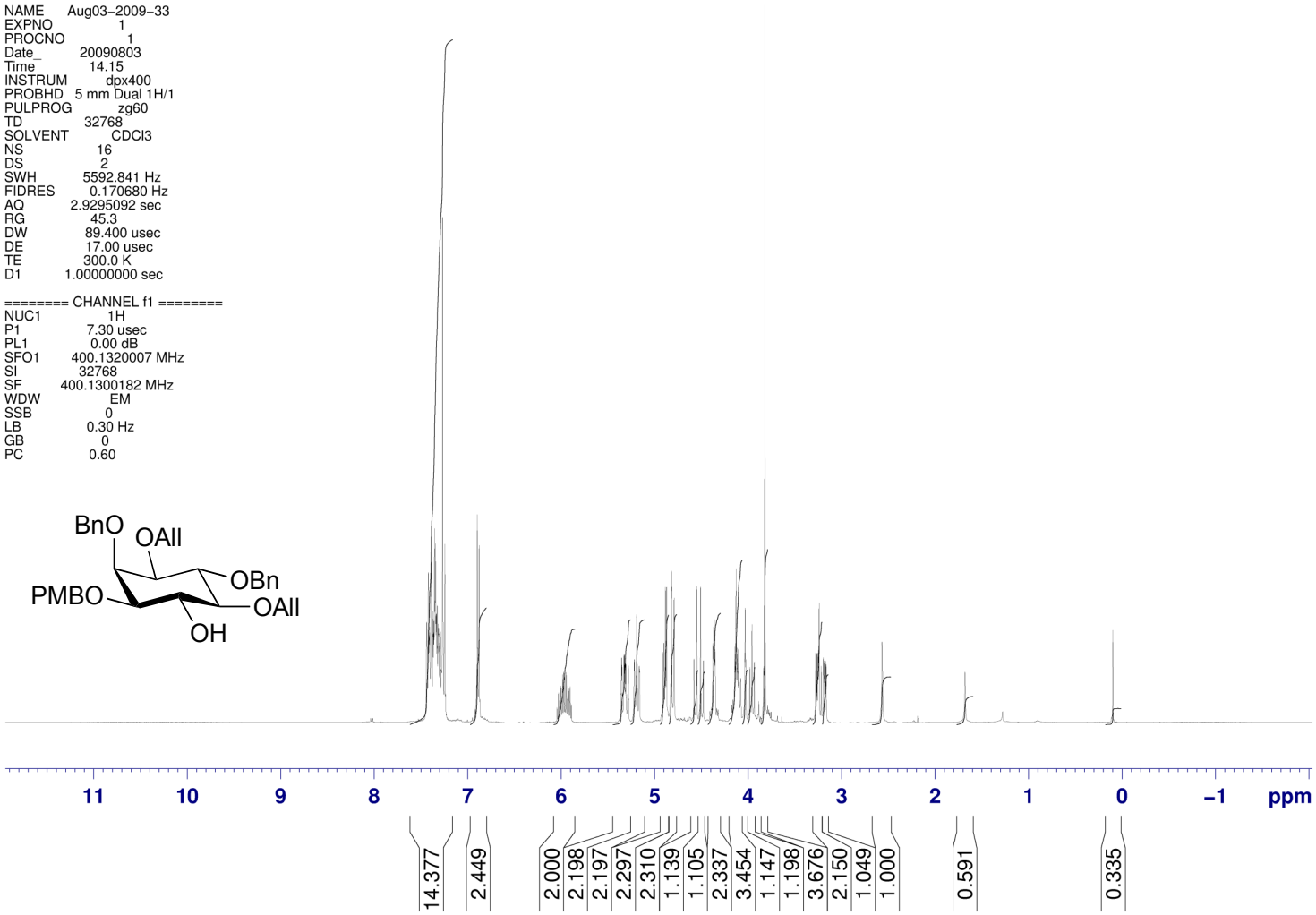

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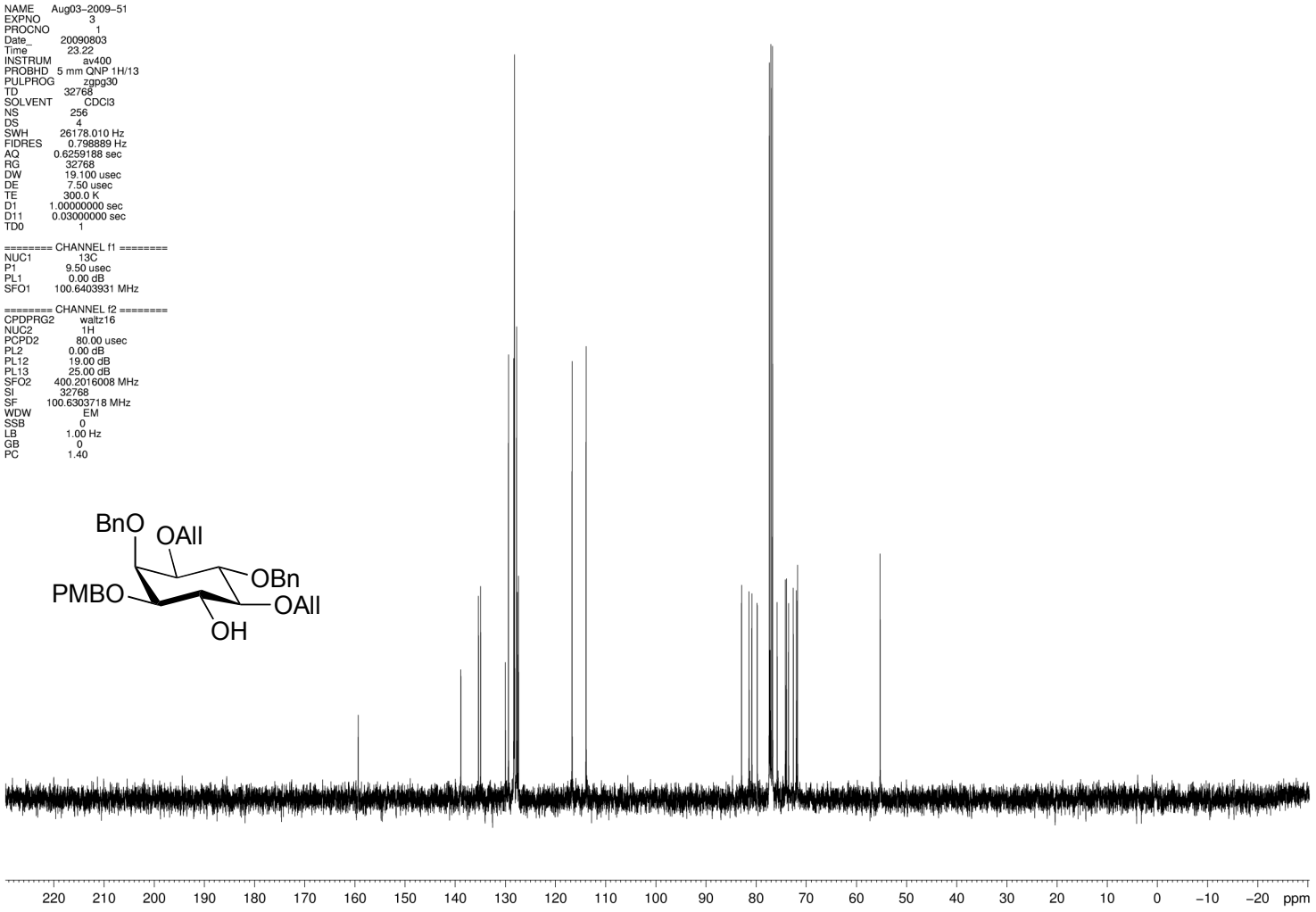

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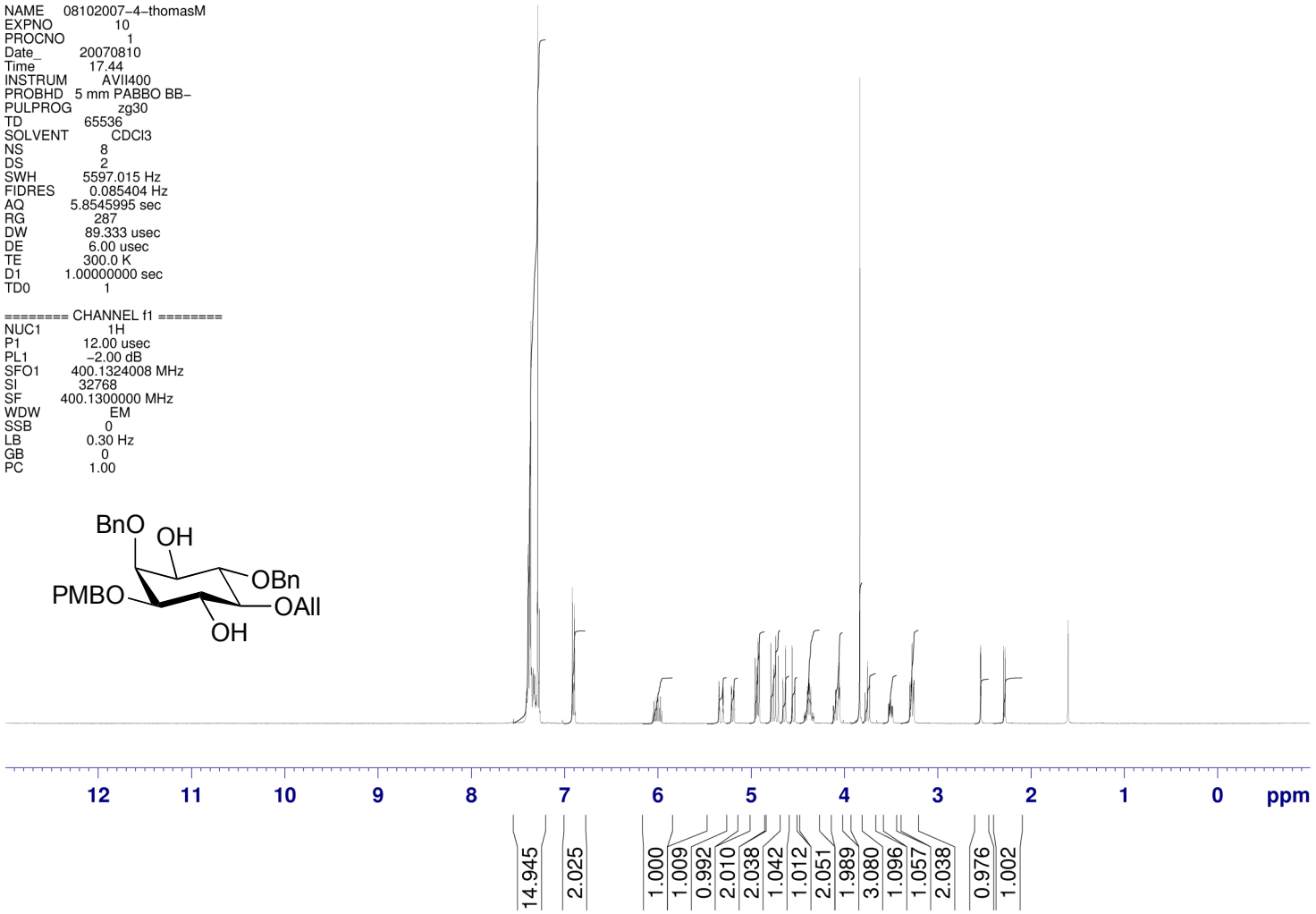
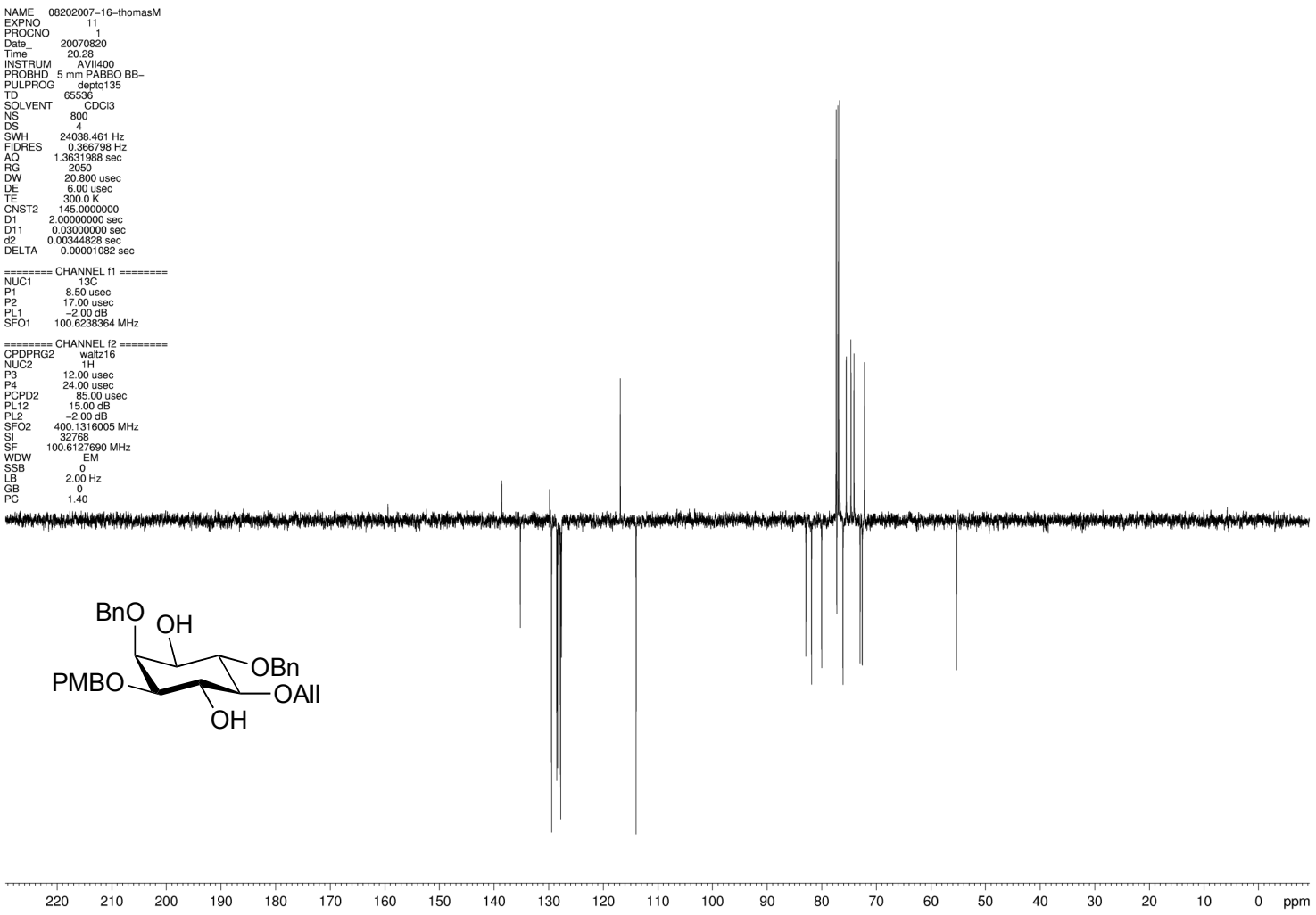
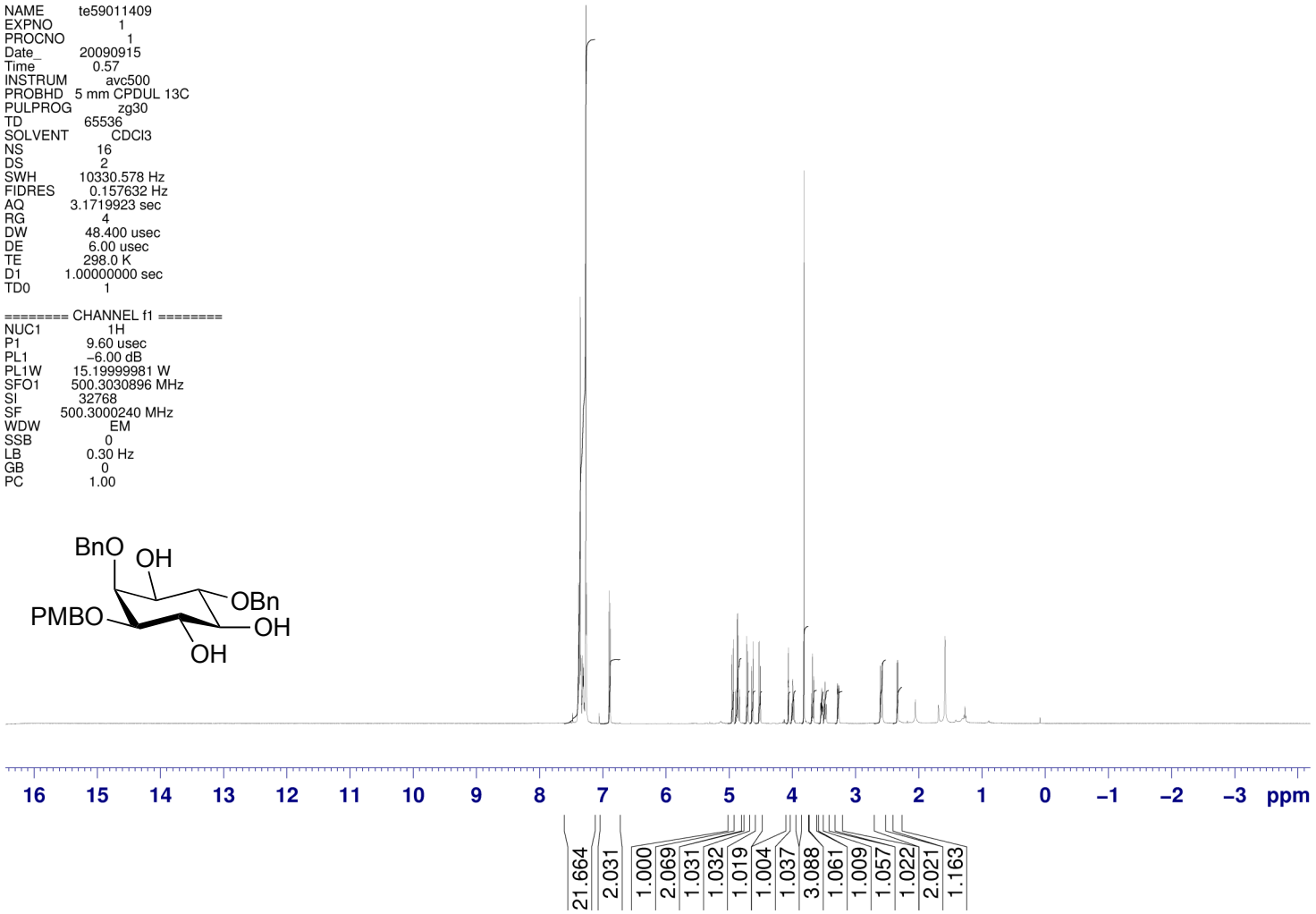


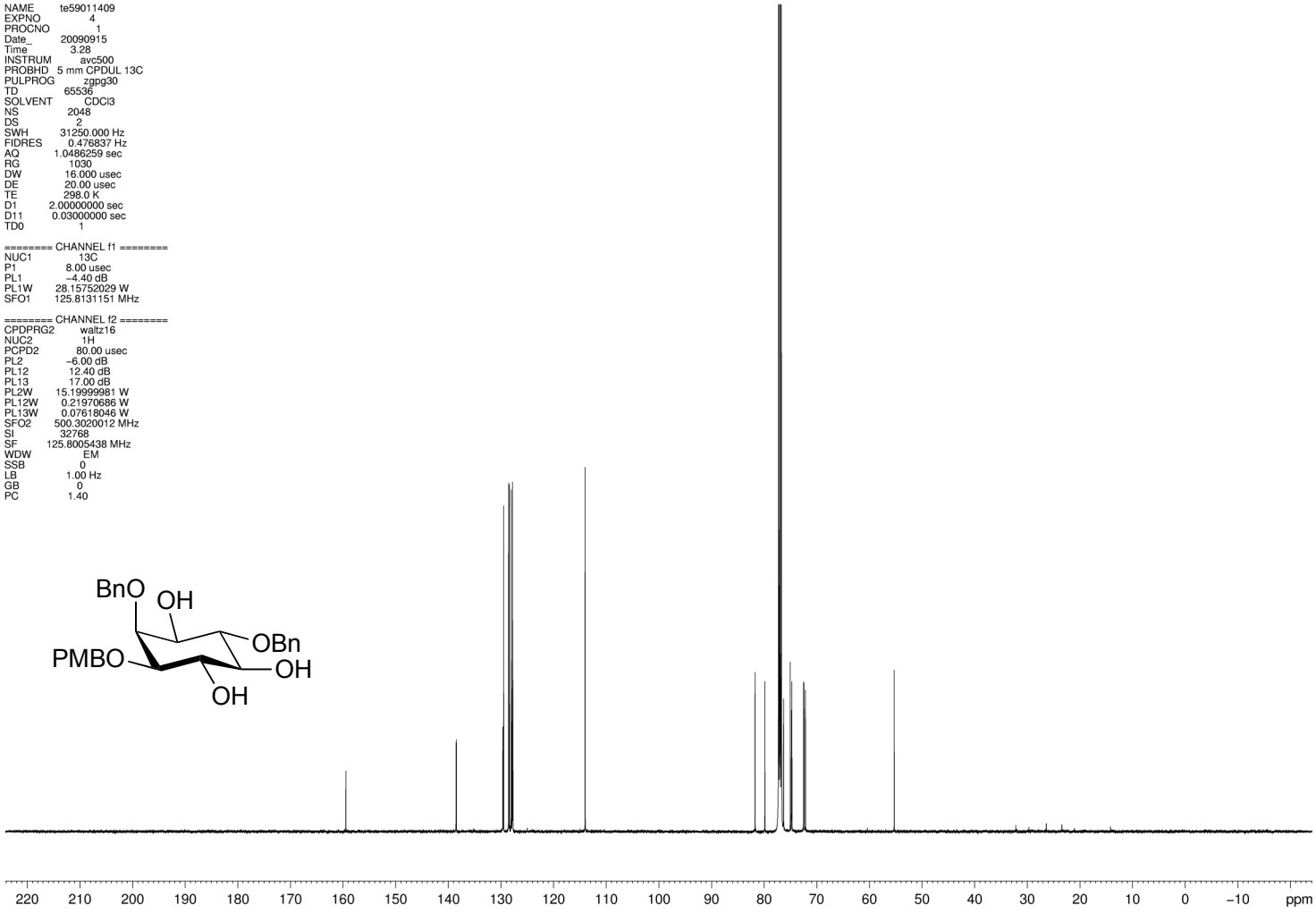
(-)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol (-)-139



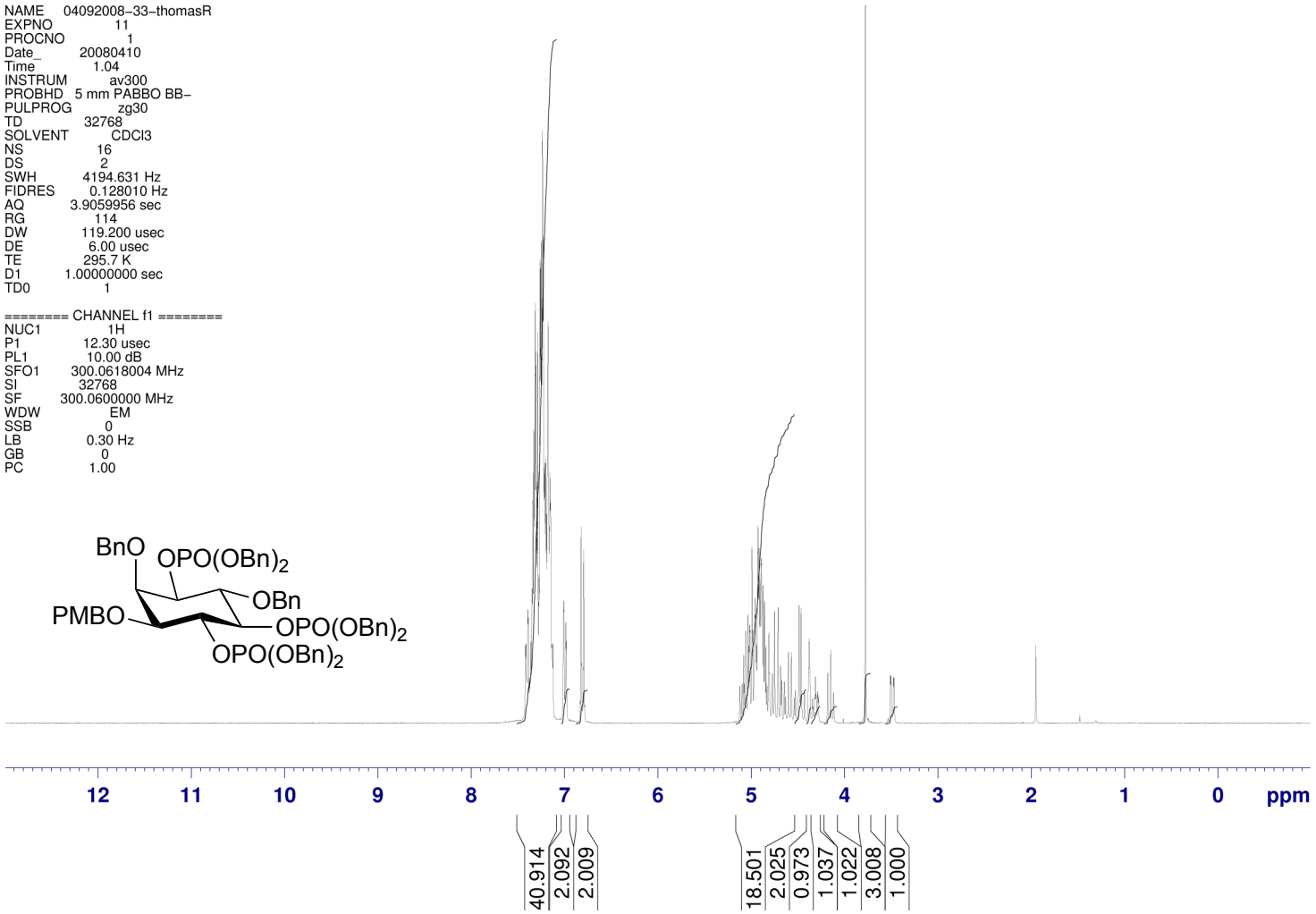
(+)-1D-2,6-bis-*O*-Benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol (+)-106



(+)-1D-2,6-bis-*O*-Benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol (+)-106



(-)-1D-2,6-bis-*O*-Benzyl-3-*O*-(4-methoxy)benzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) (-)-107



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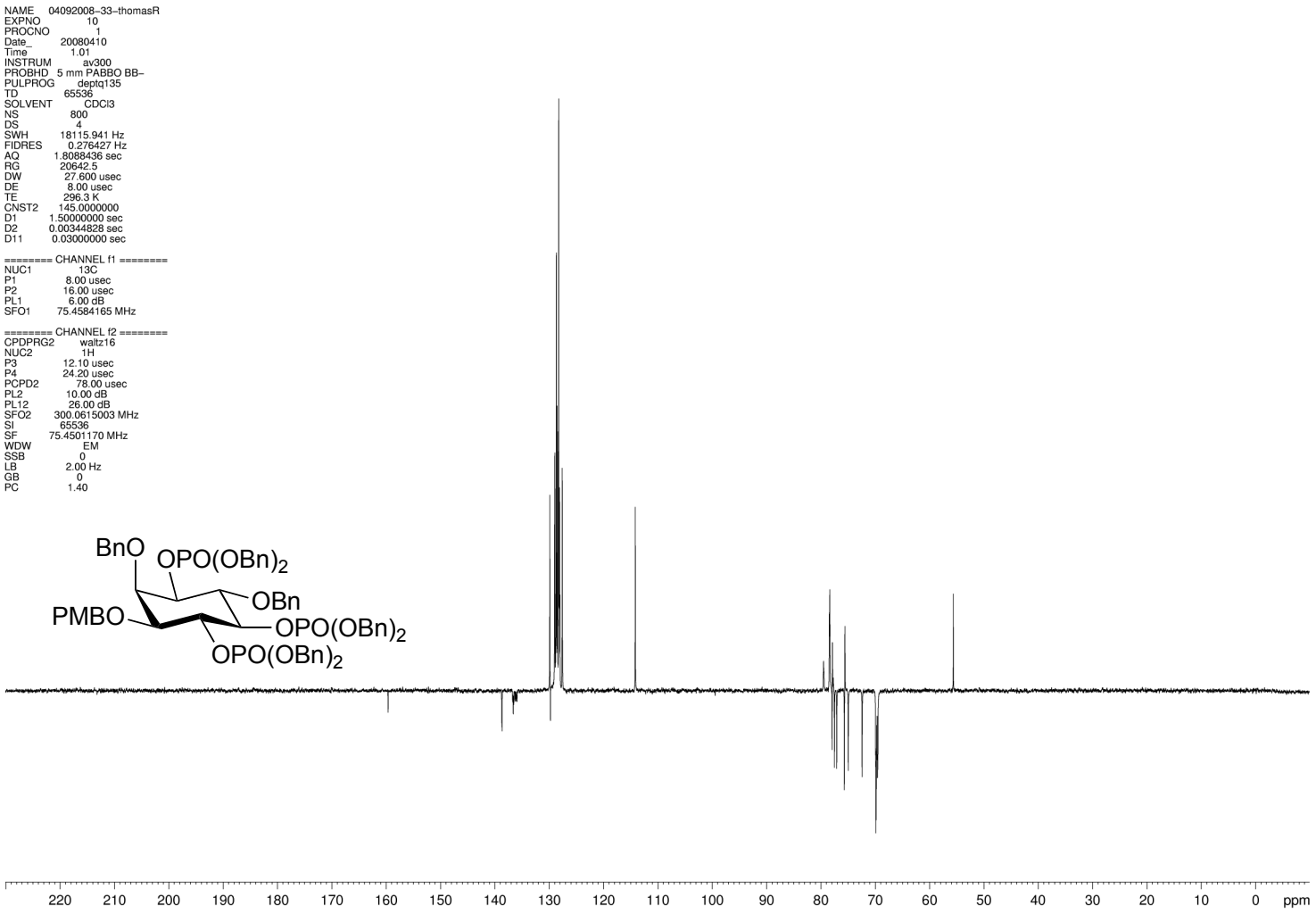

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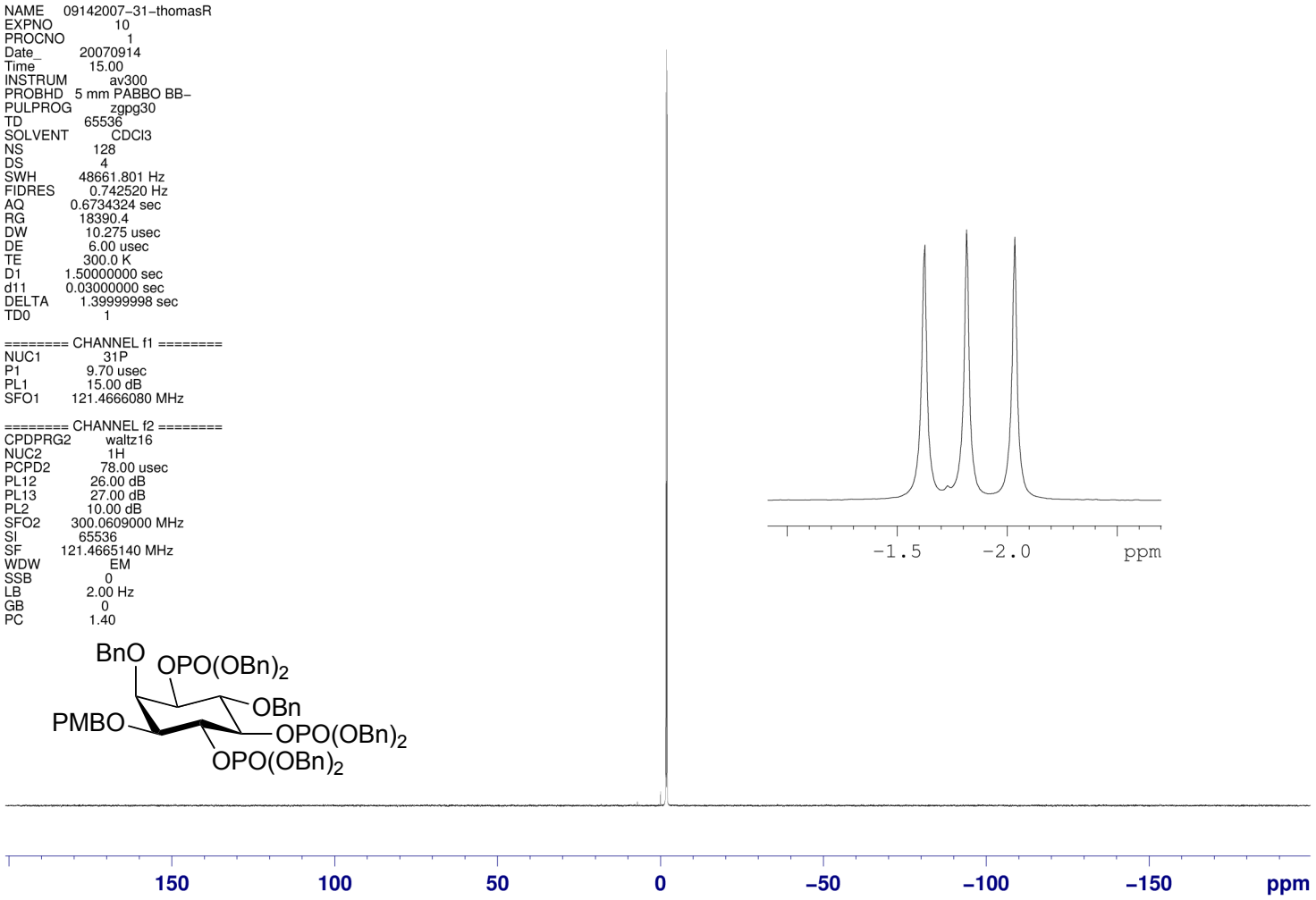

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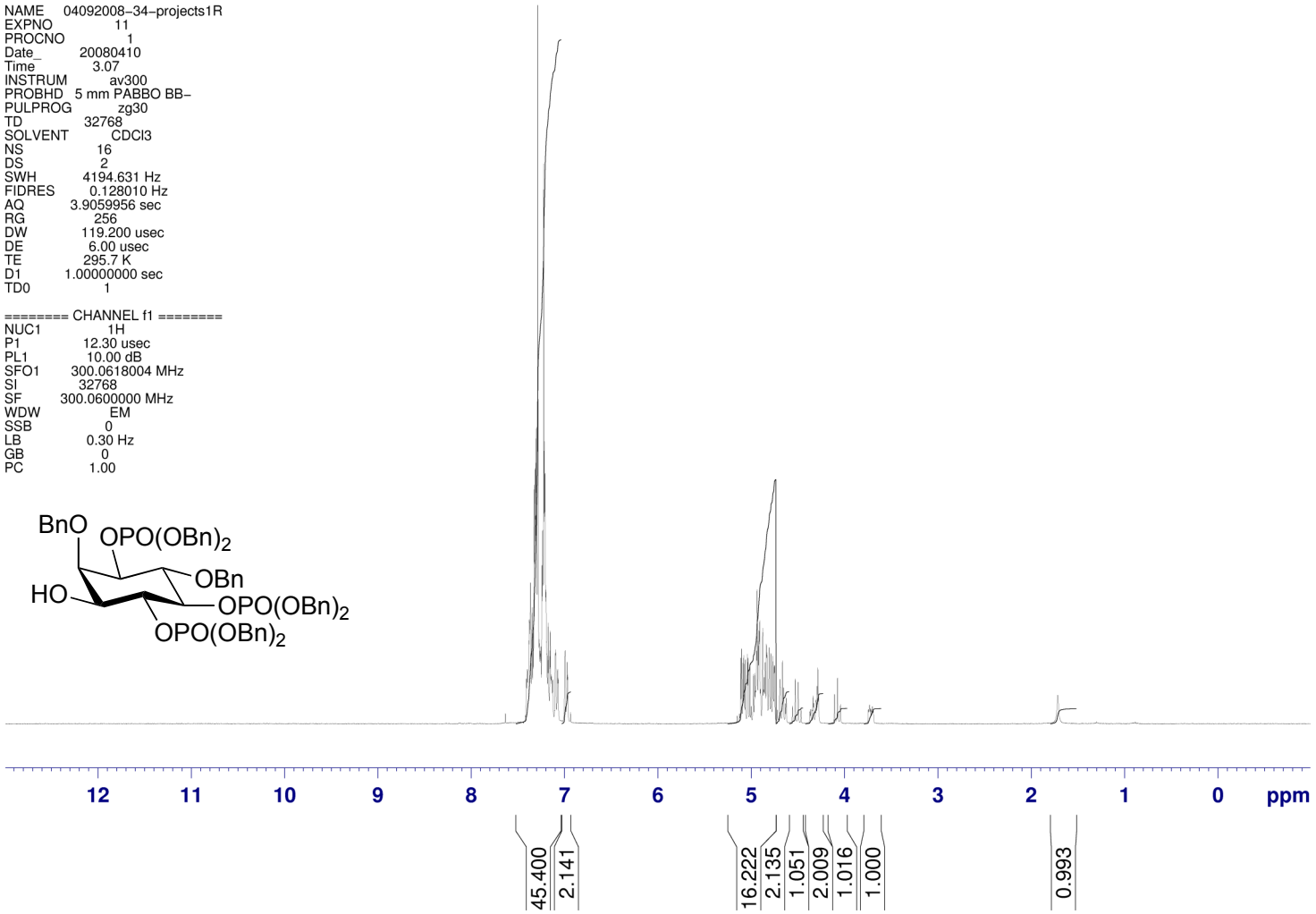

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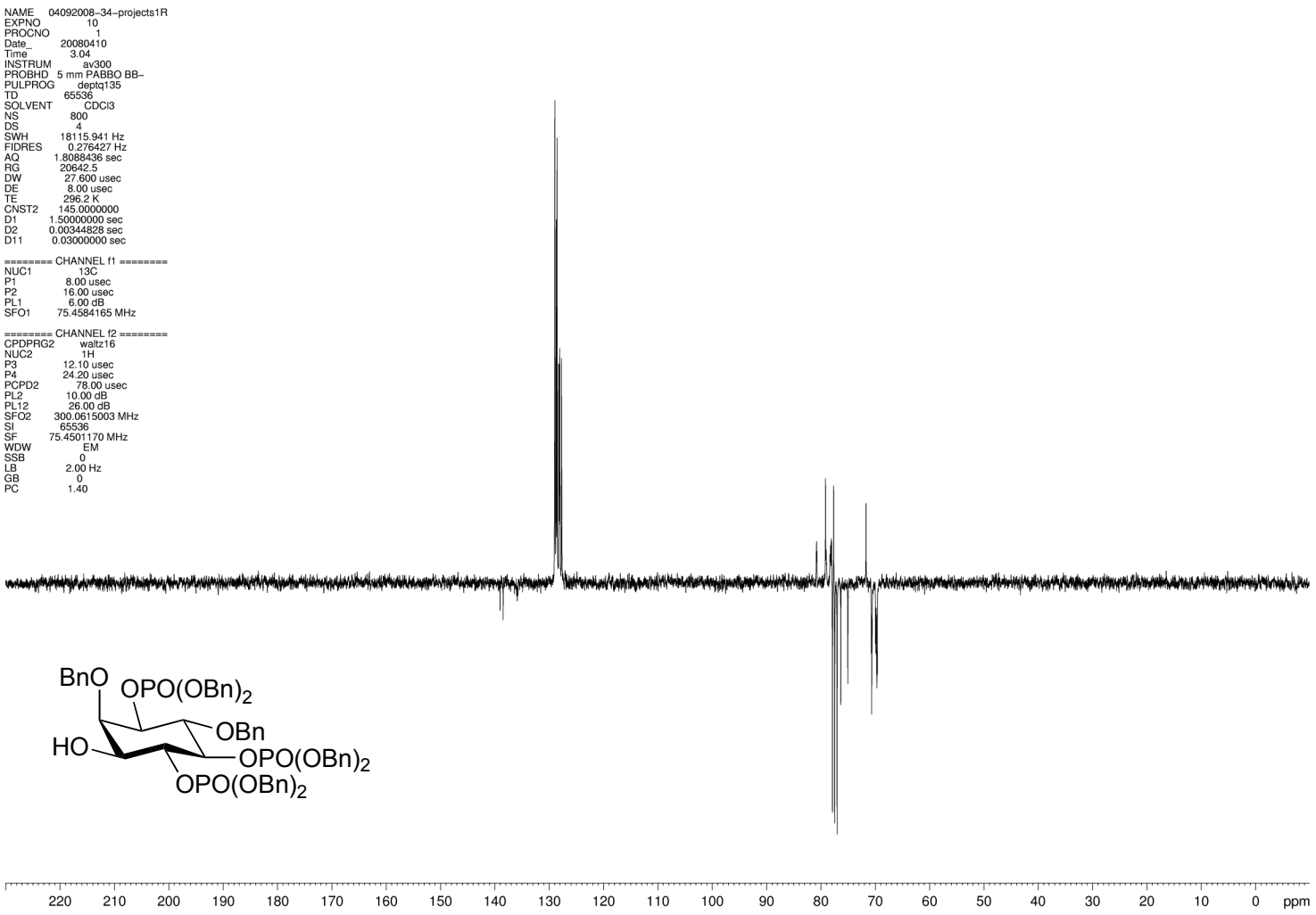

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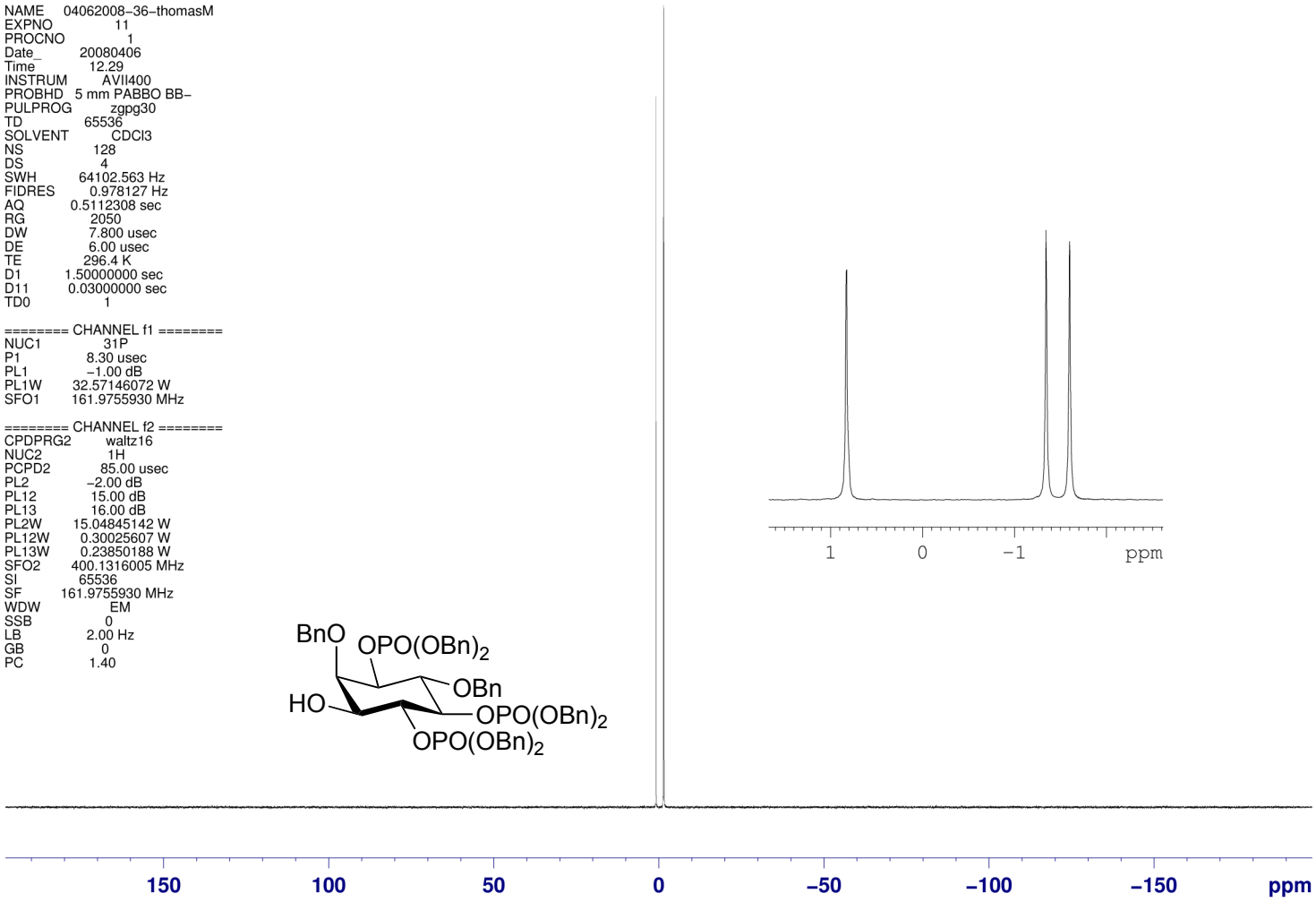
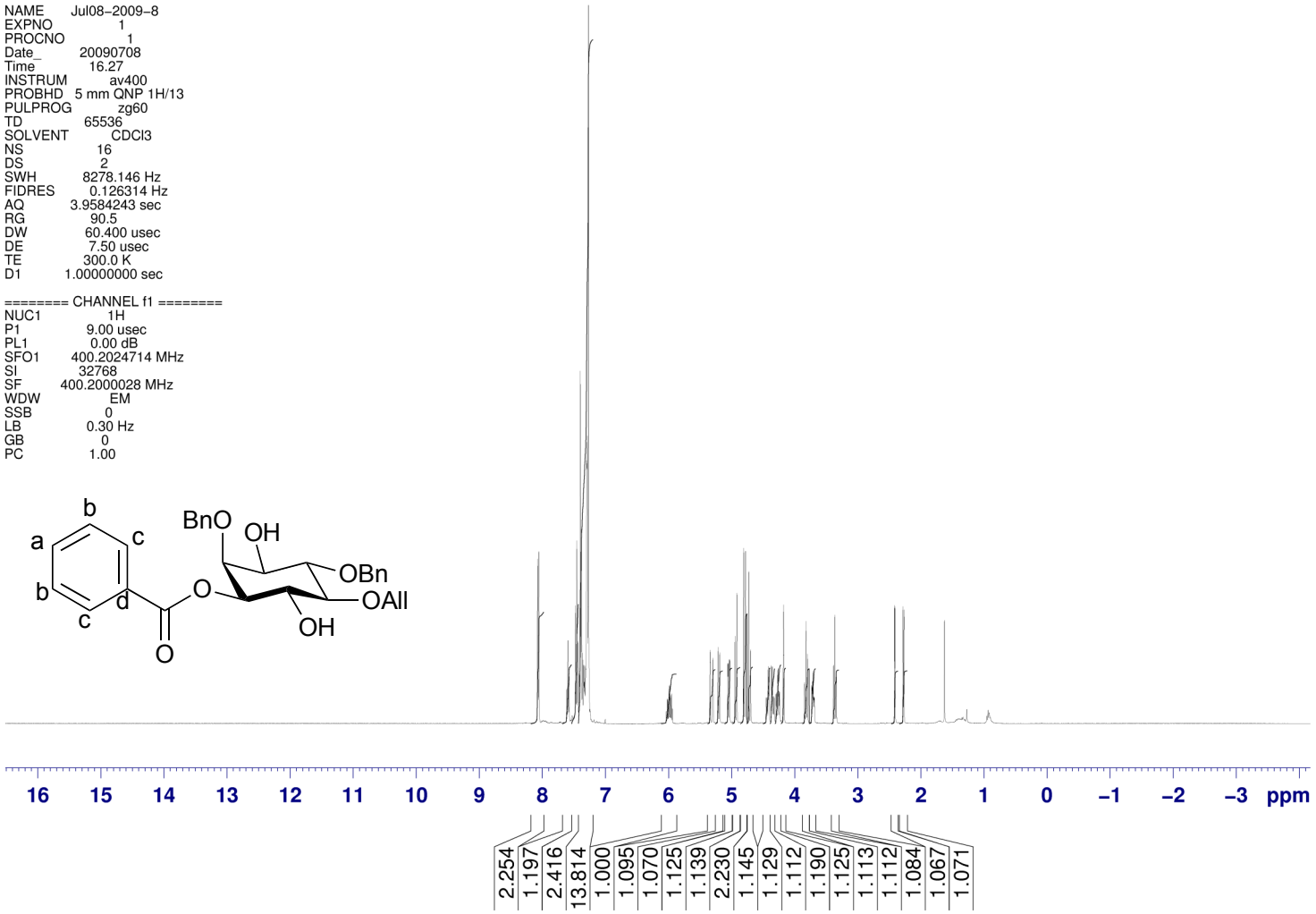


(±)-1D-5-*O*-Allyl-2,6-bis-*O*-benzyl-3-*O*-benzoyl-*myo*-inositol 150



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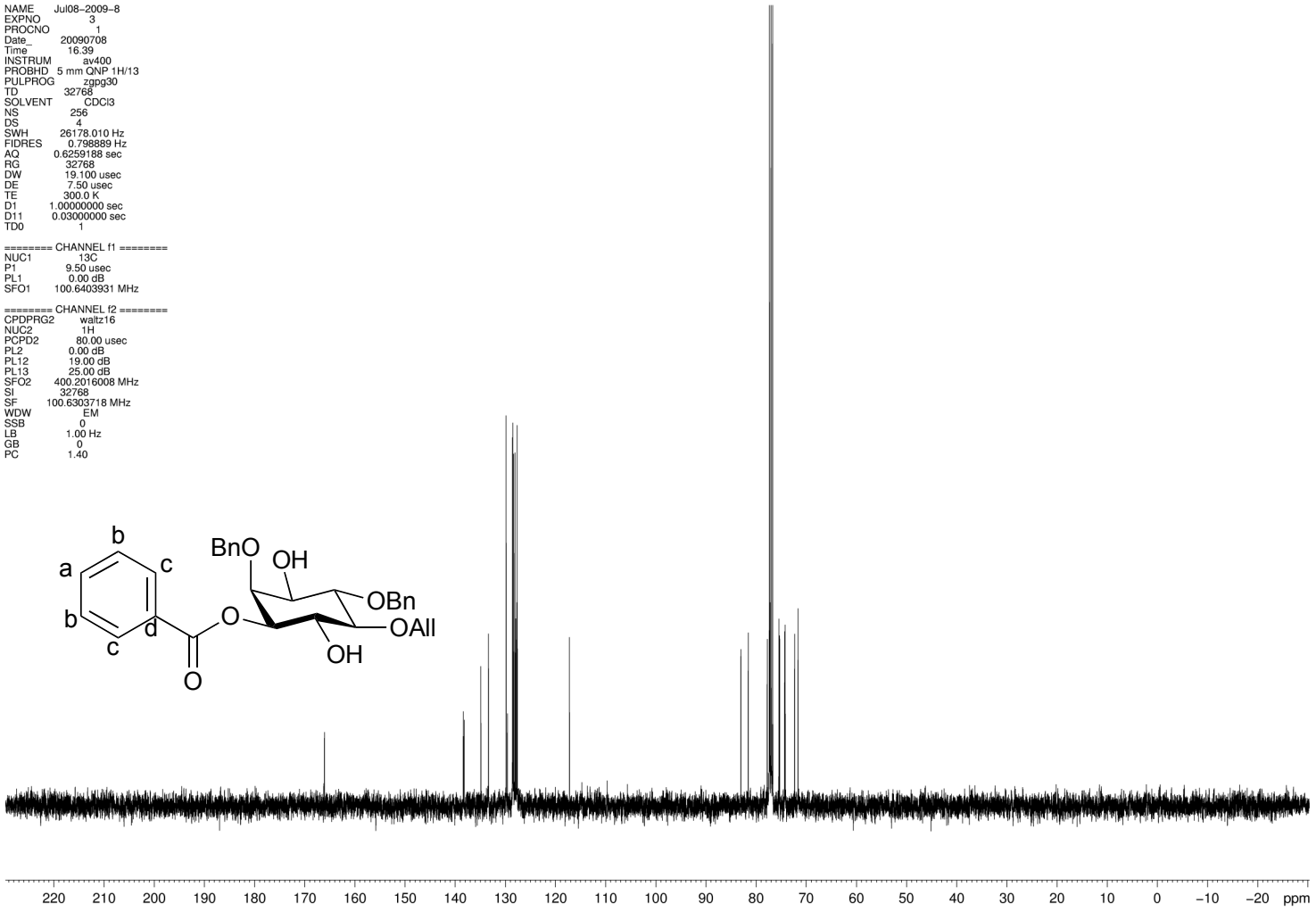

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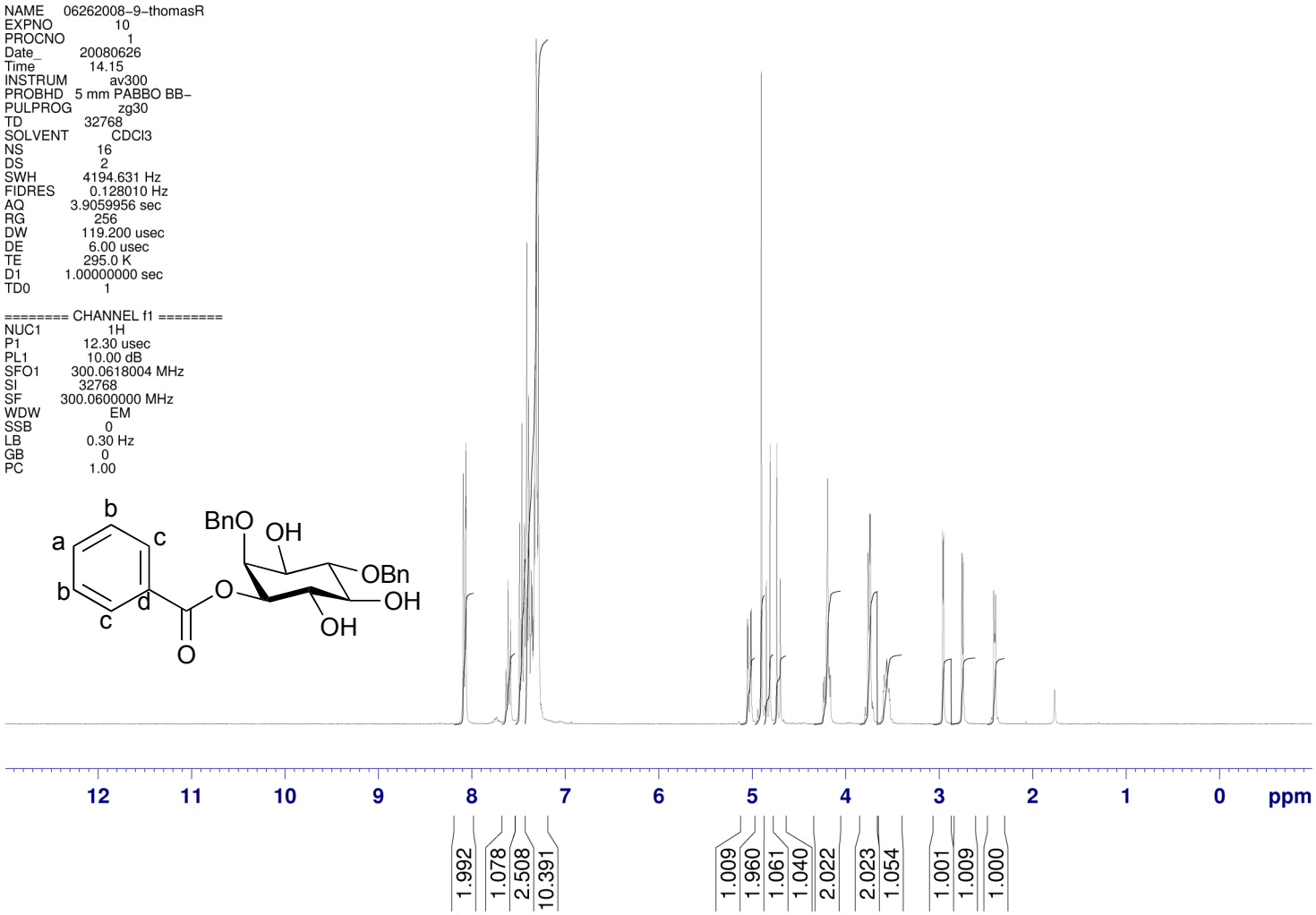

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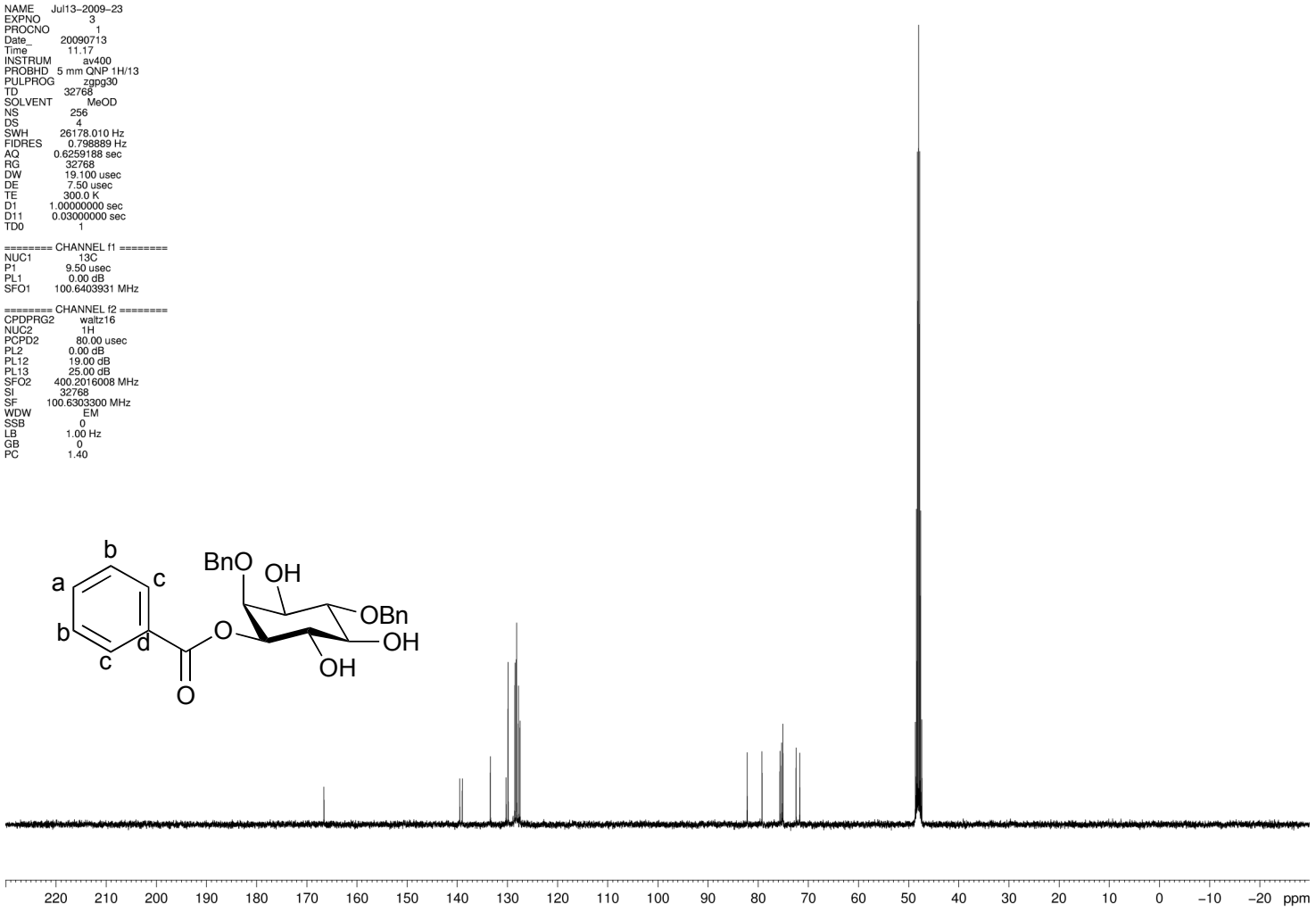

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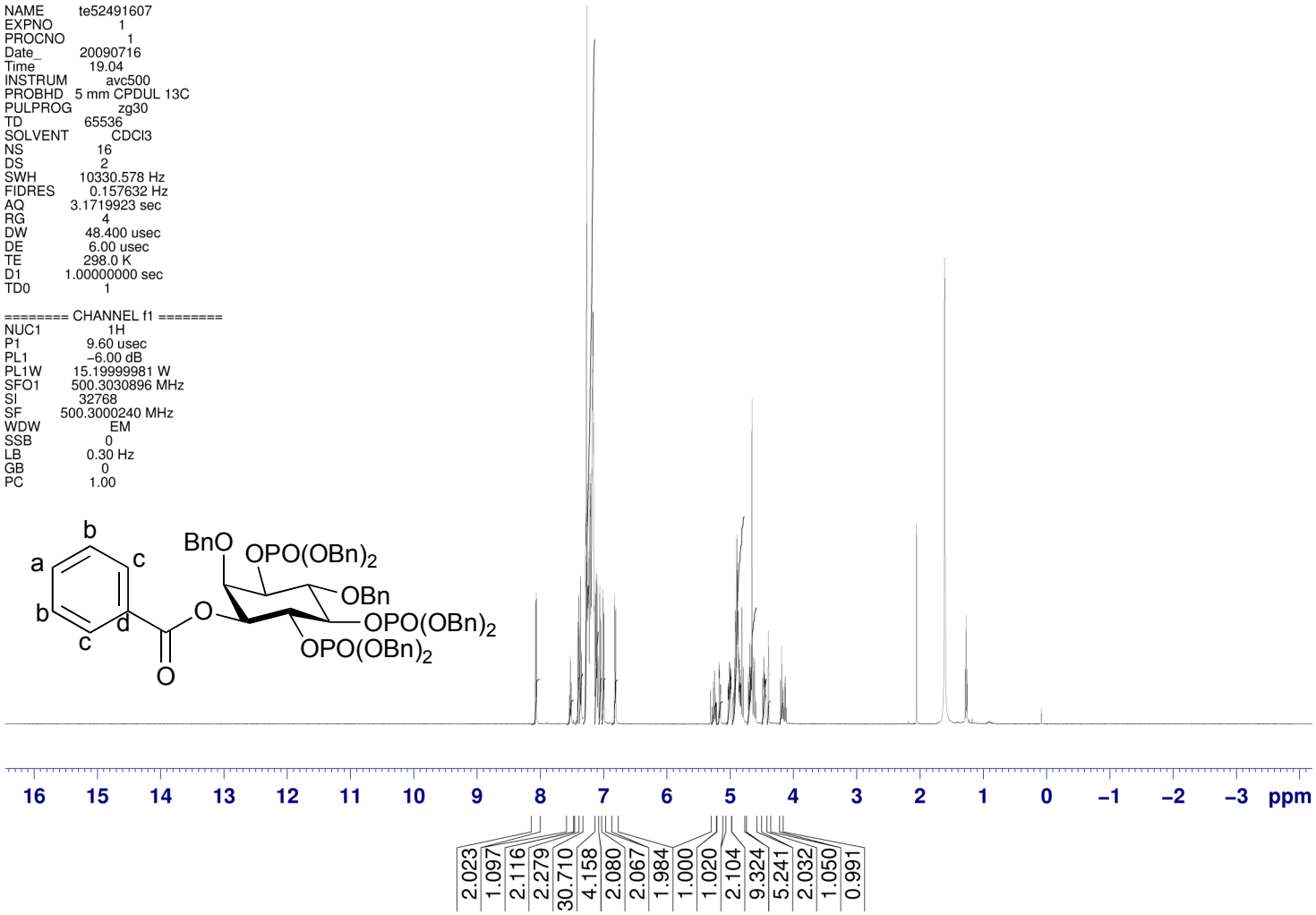

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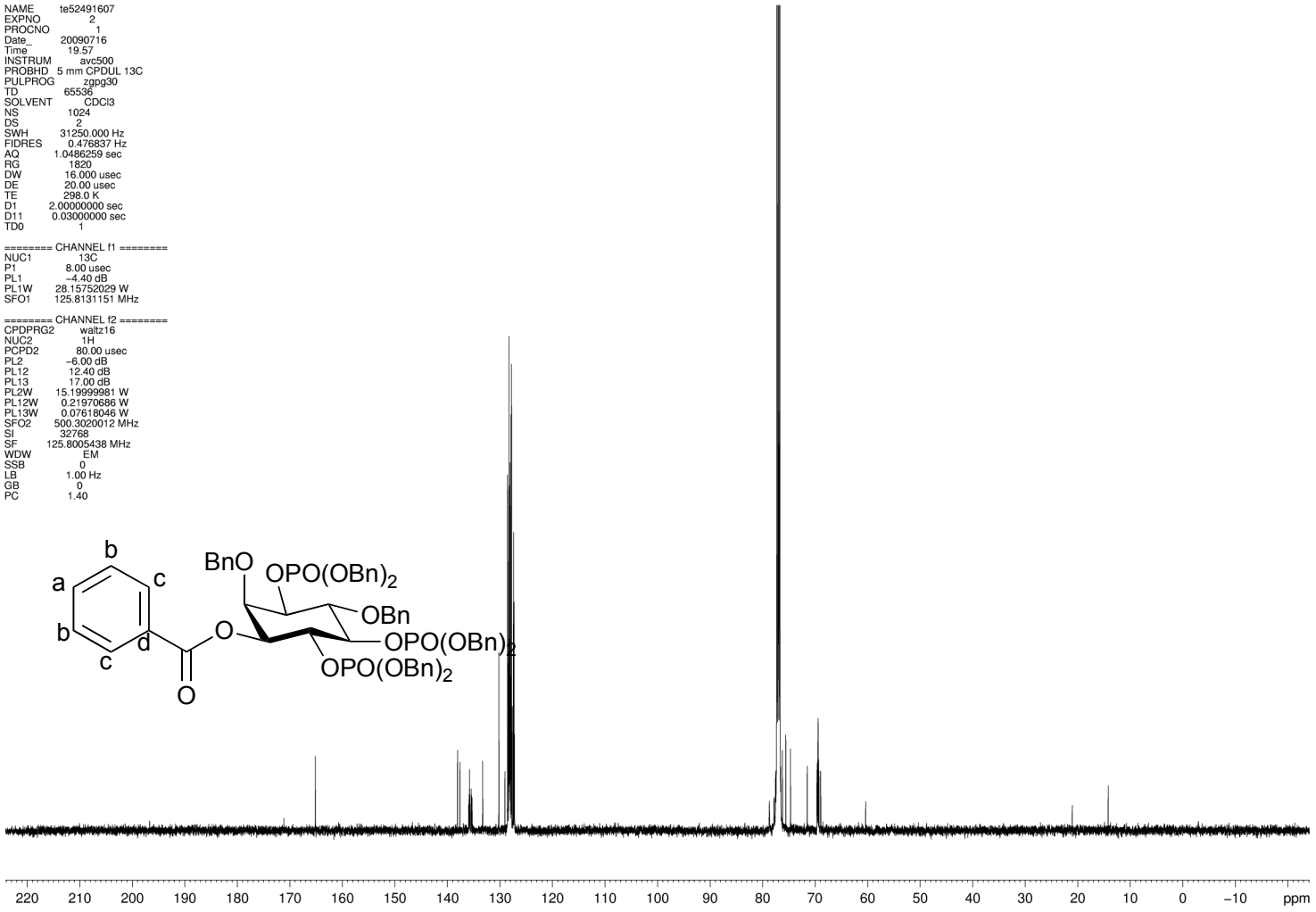

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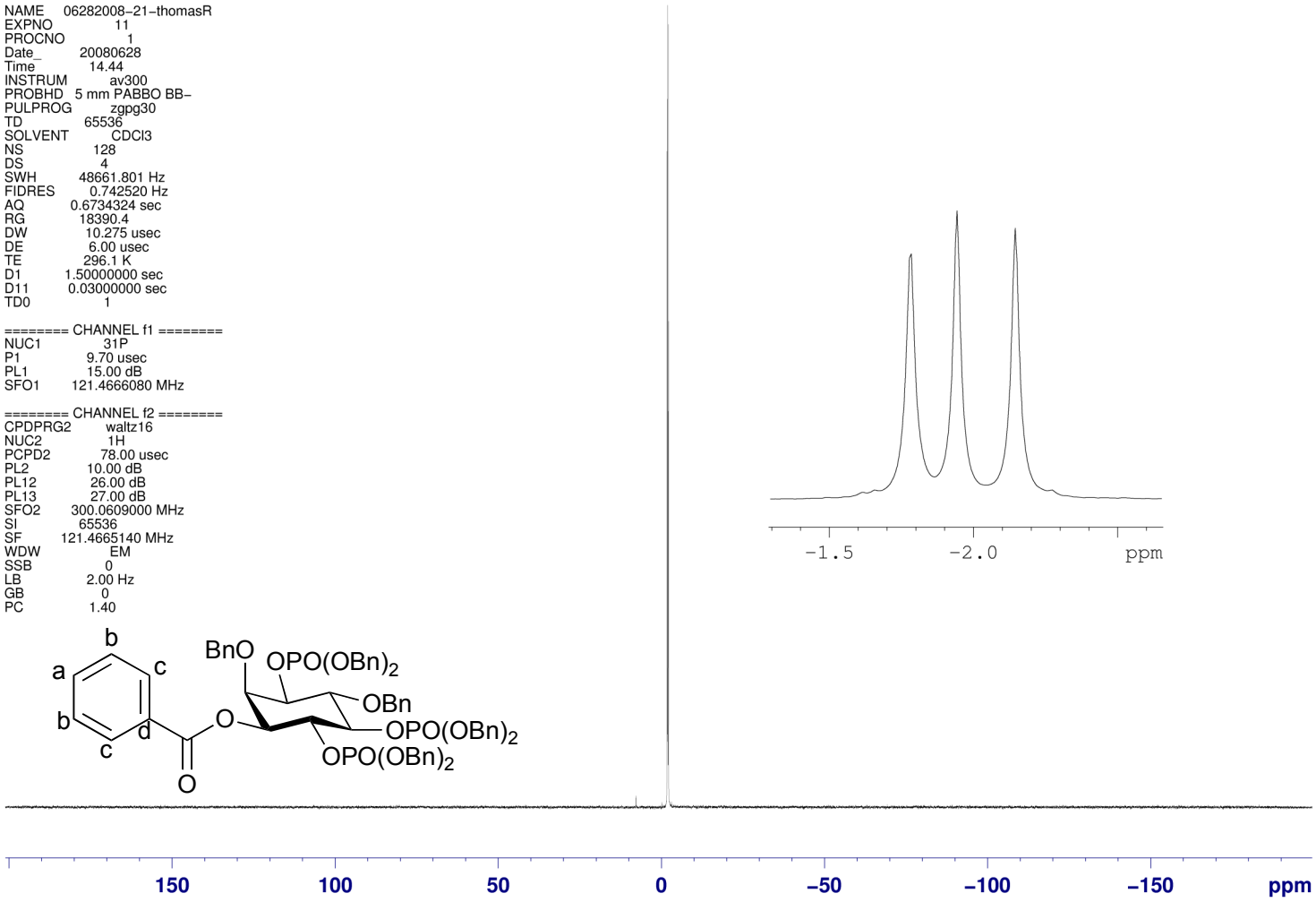
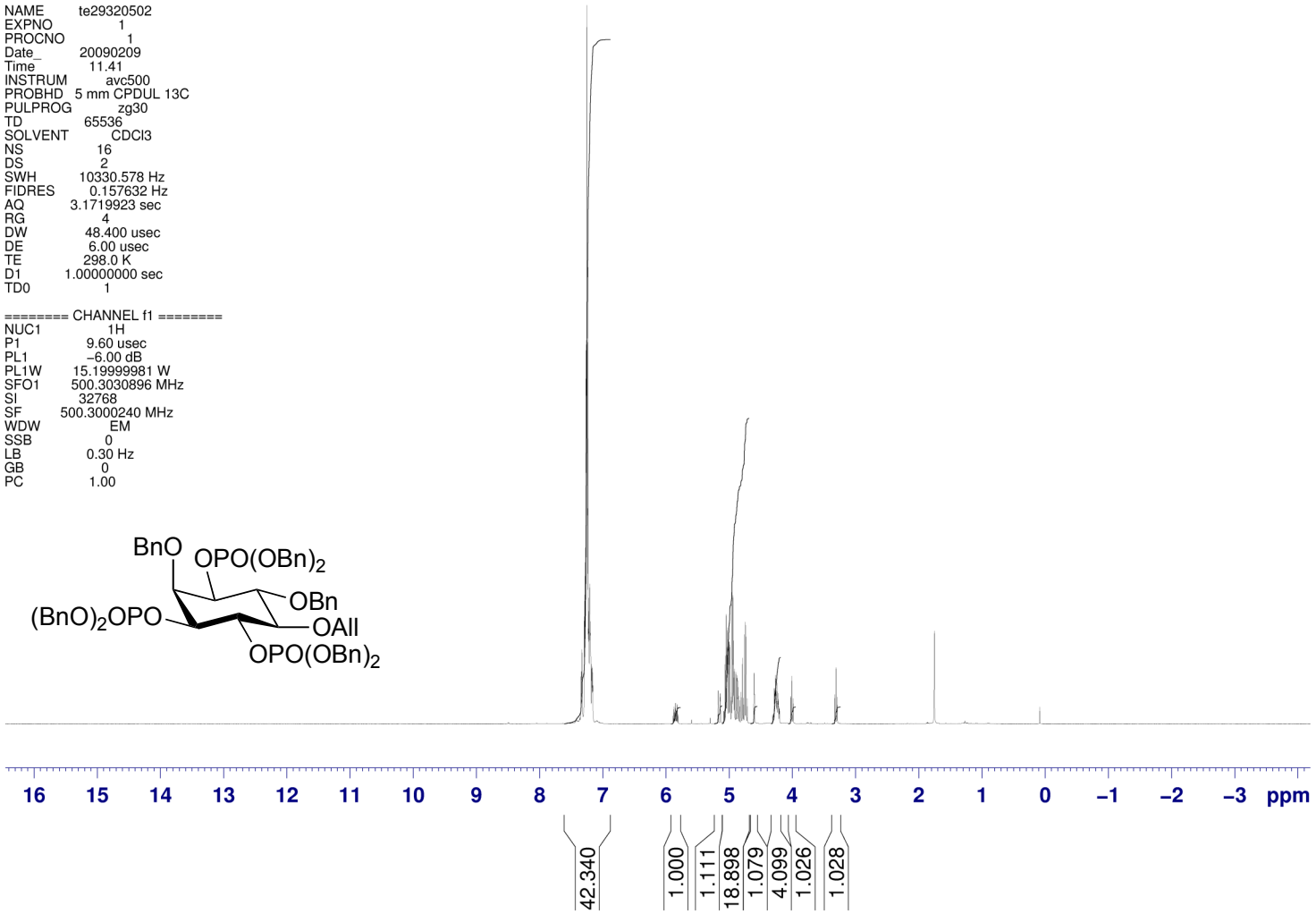
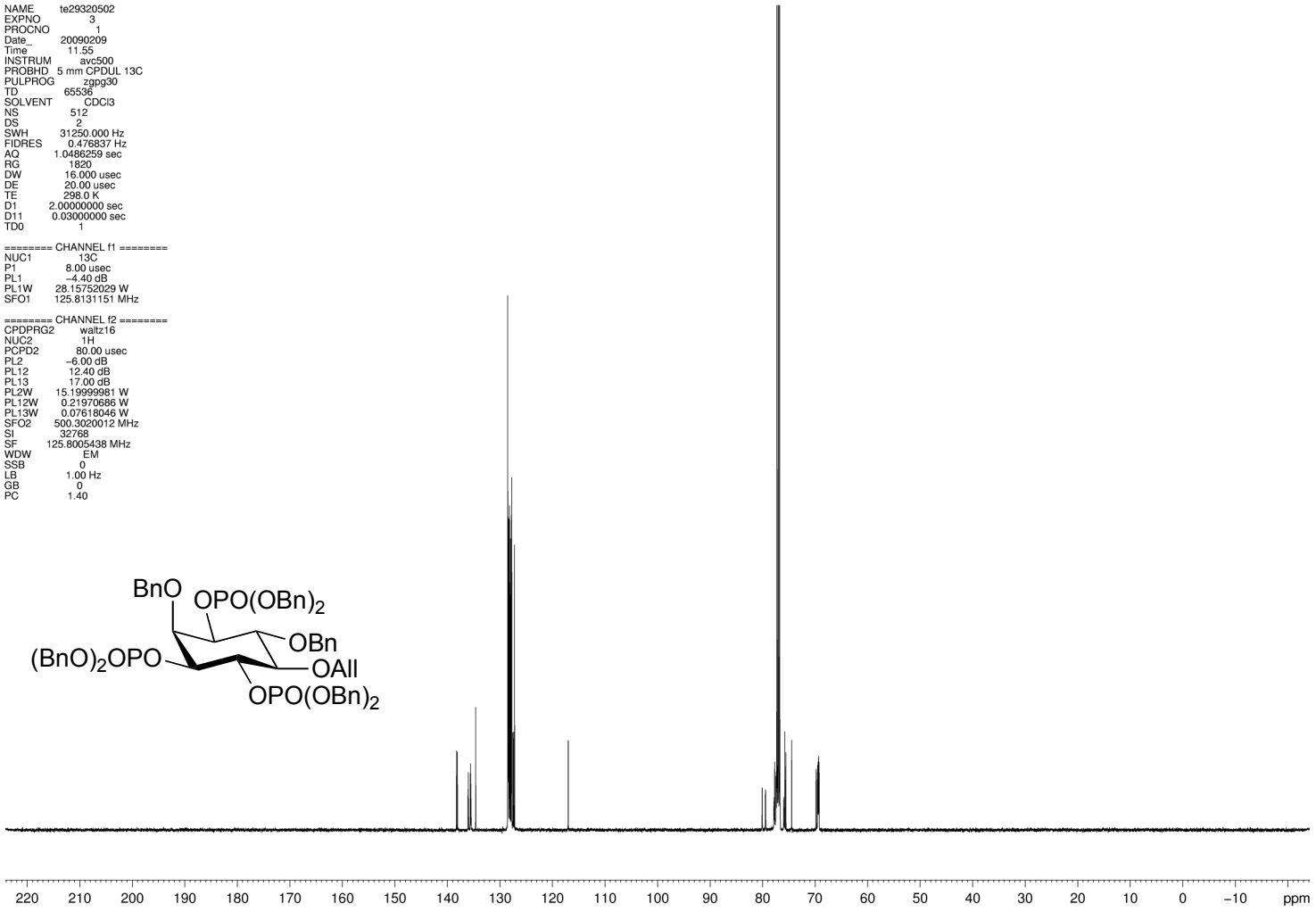


(+)-1D-2,6-bis-*O*-Benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 152



(+)-1D-2,6-bis-*O*-Benzyl-5-*O*-allyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate) 152



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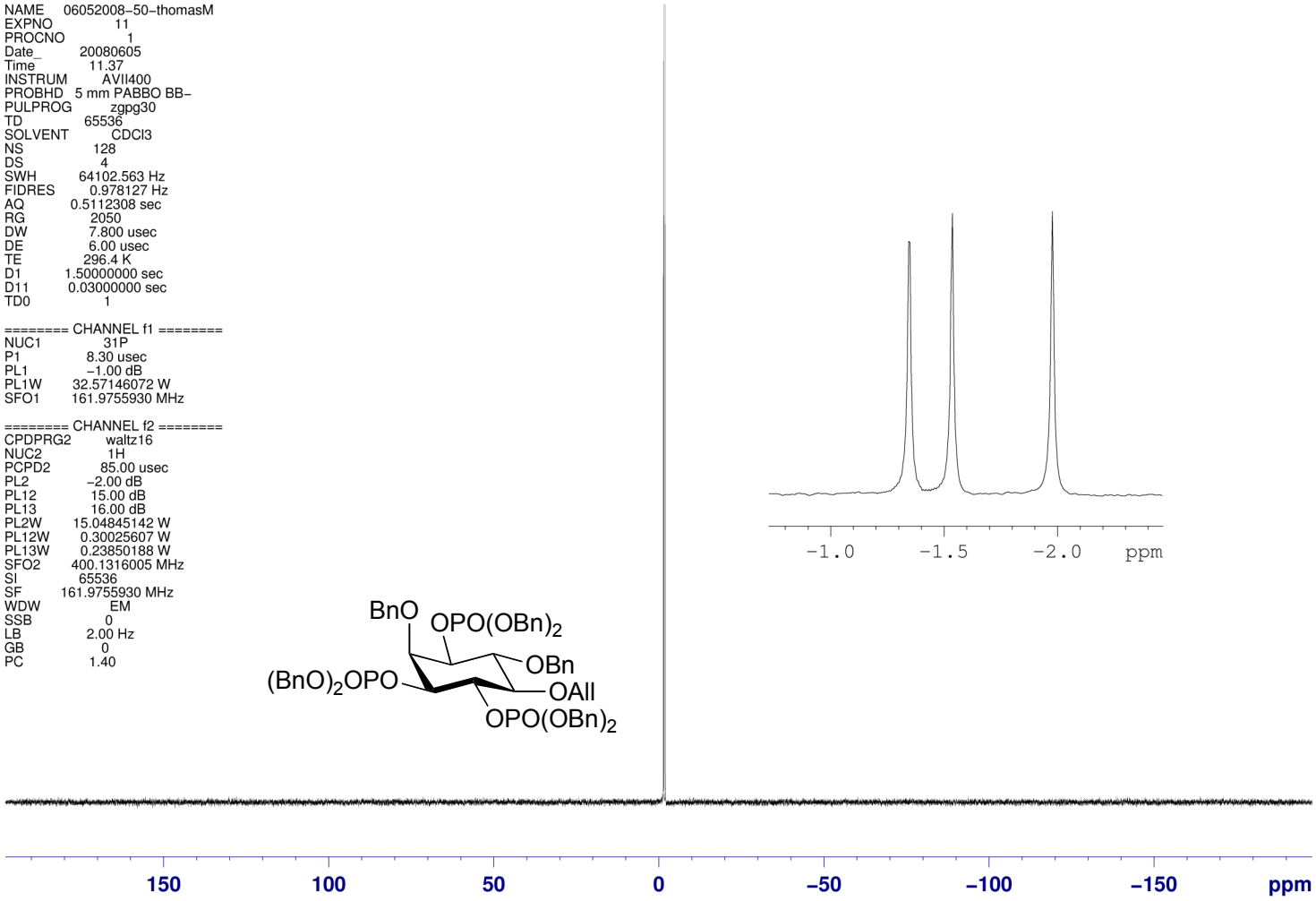

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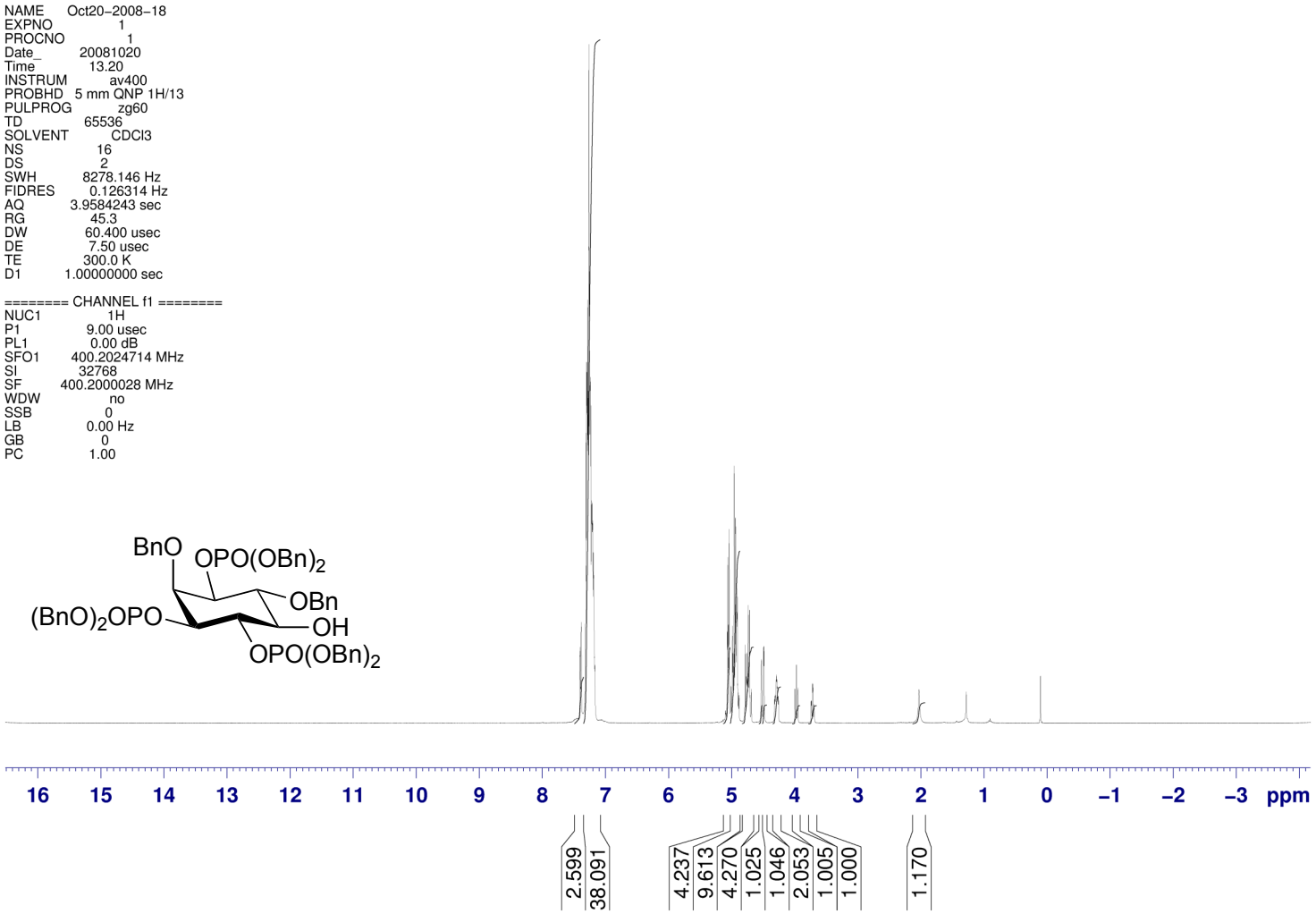

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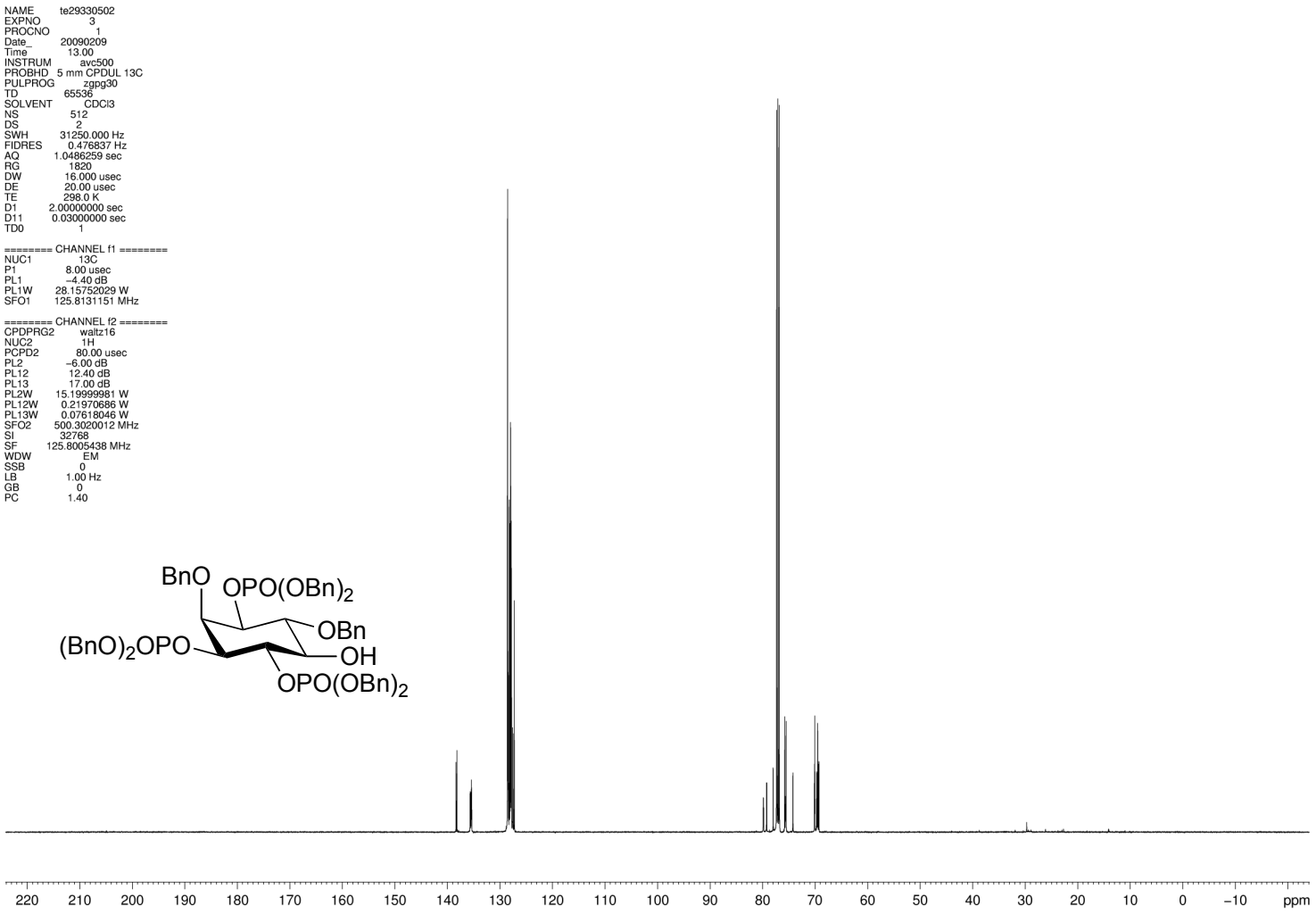

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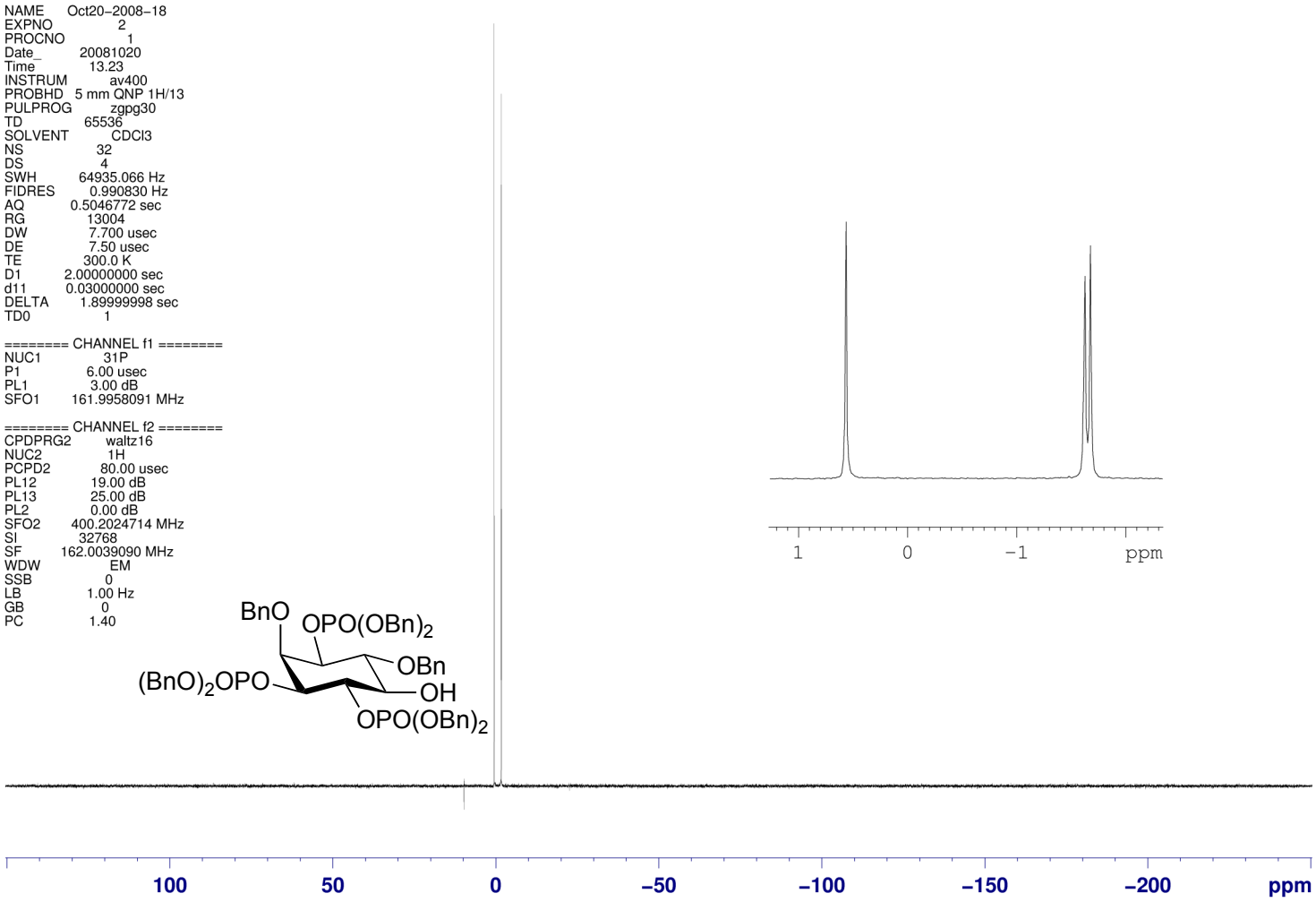

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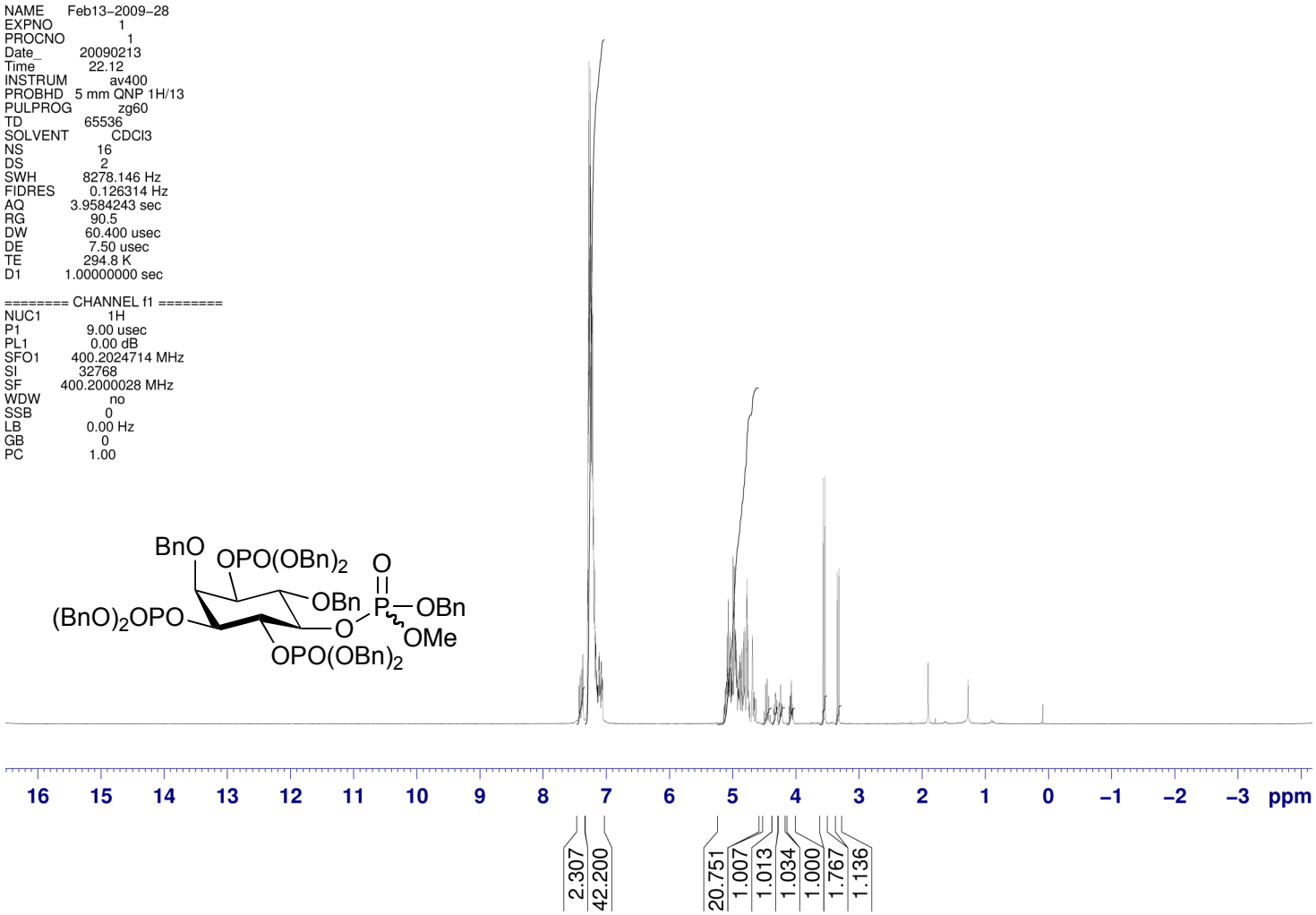
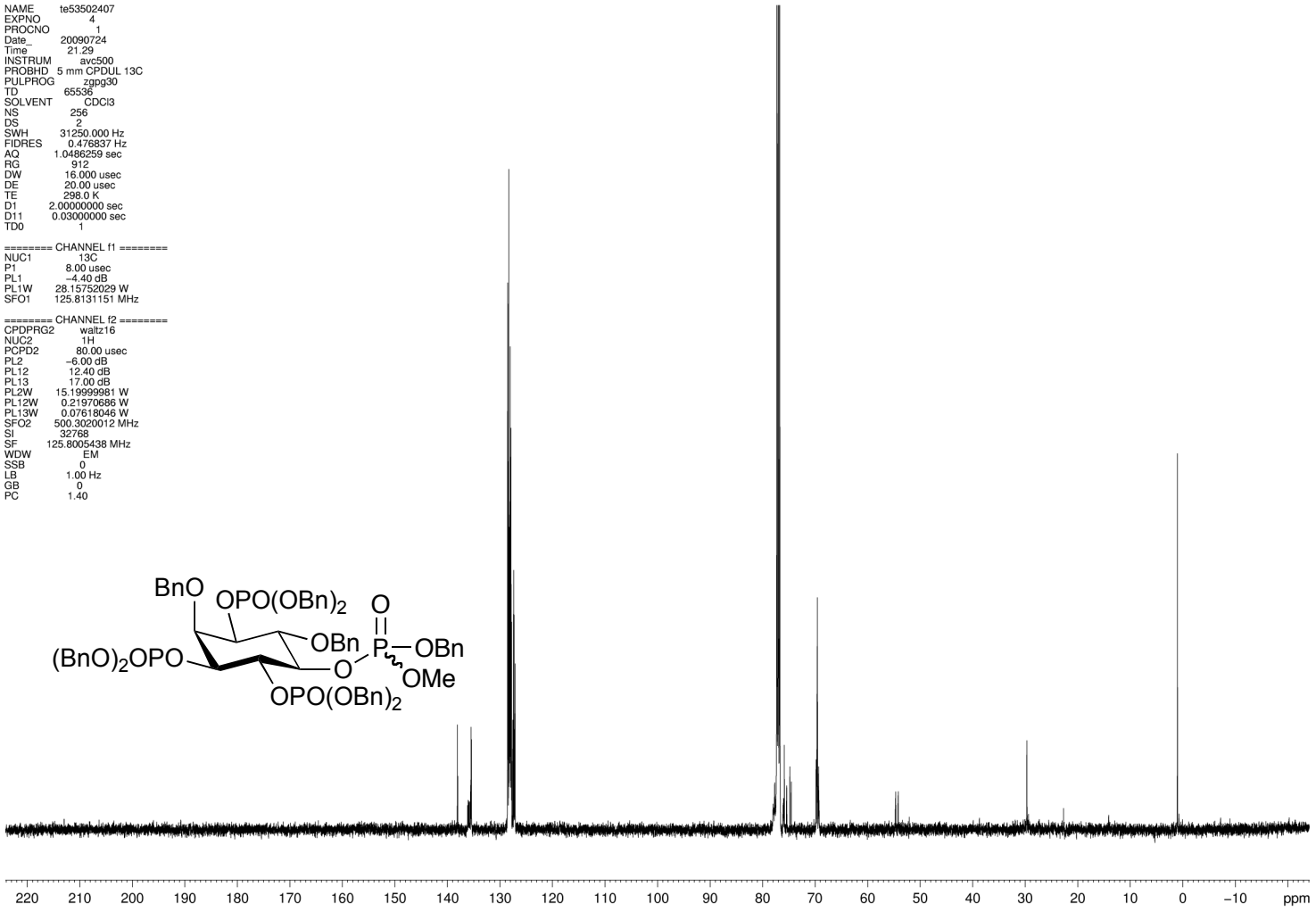


(-)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 5-(*O*-benzyl-*O*-methyl)phosphate-1,3,4-tris(dibenzylphosphate) 156



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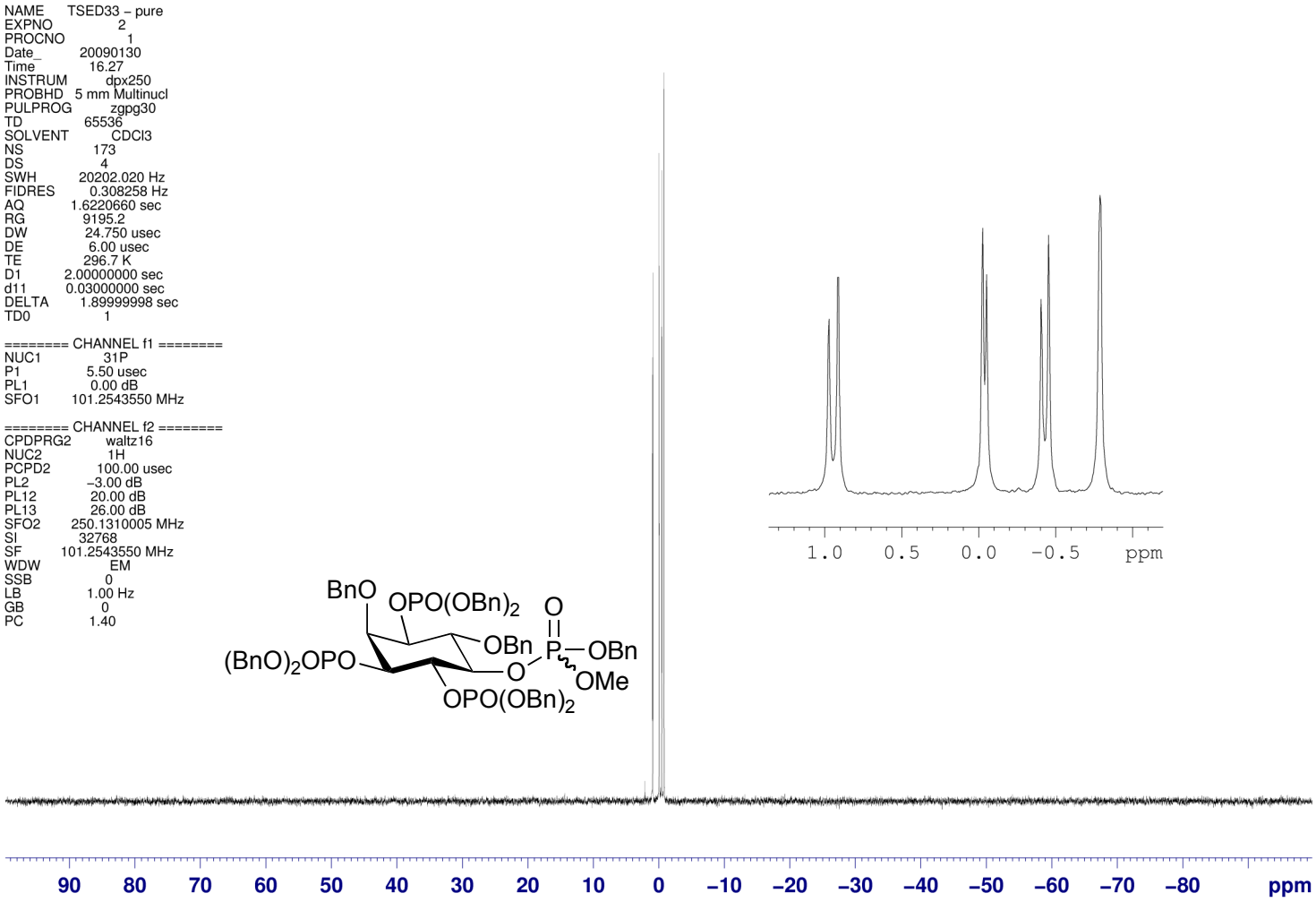
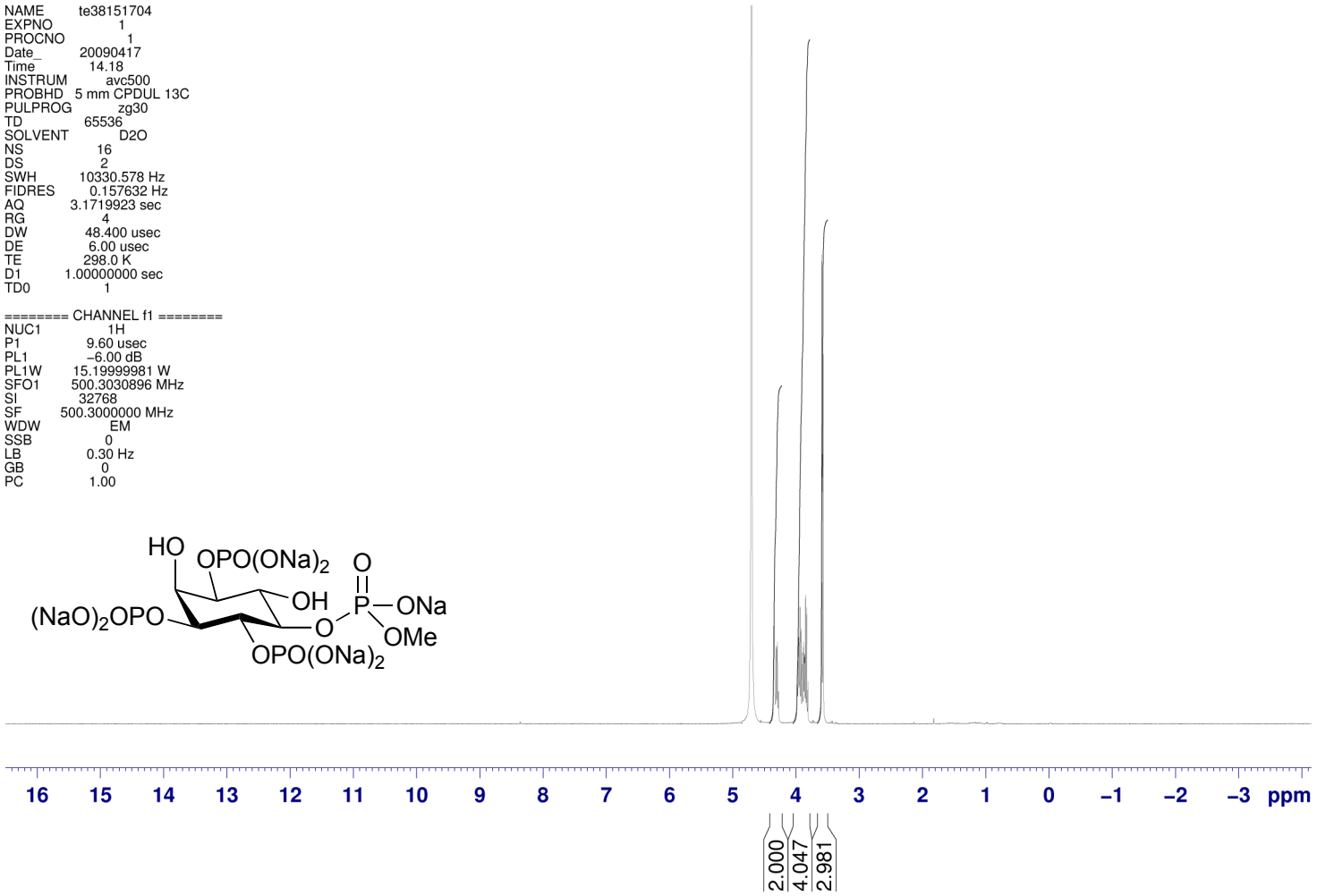
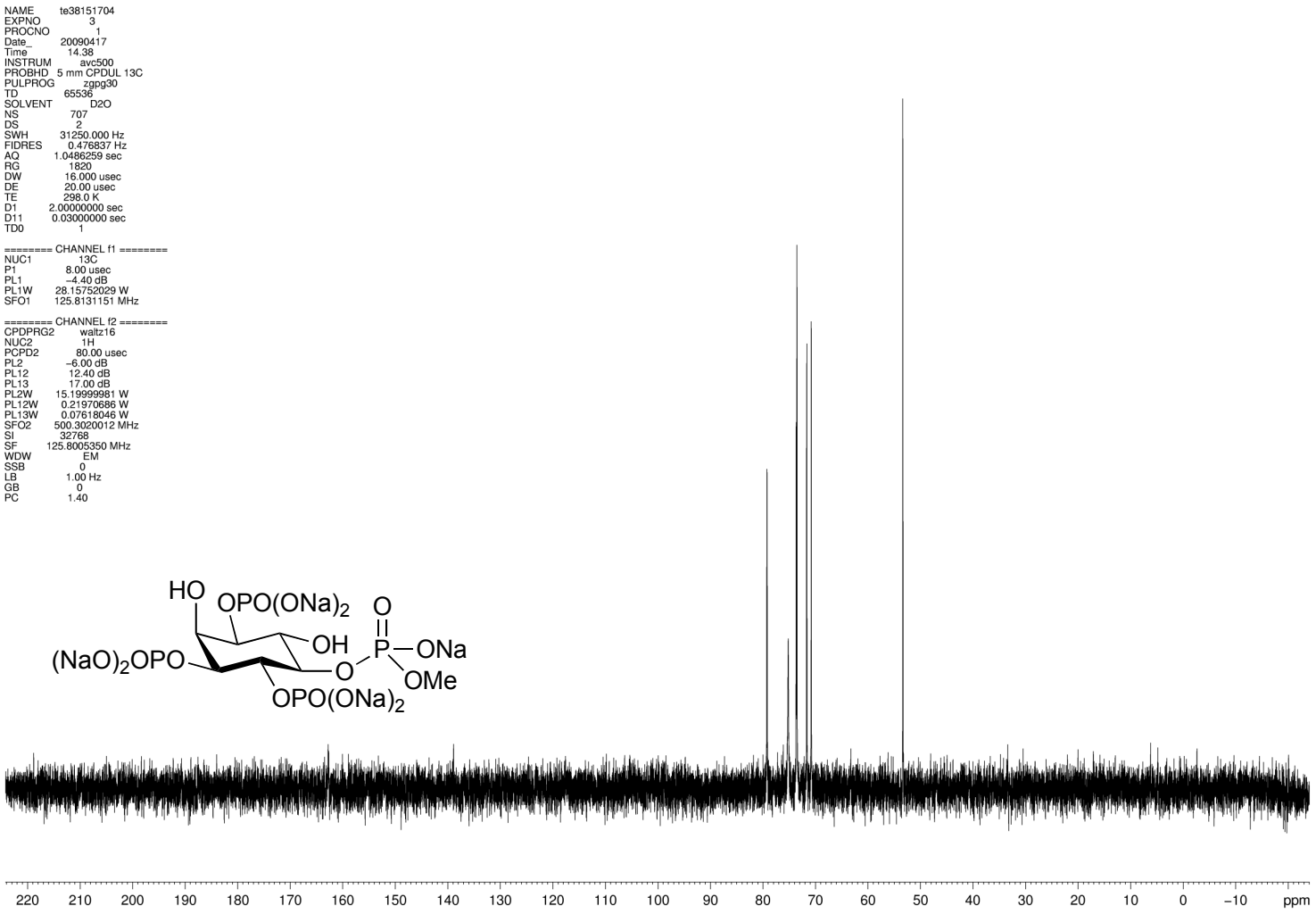


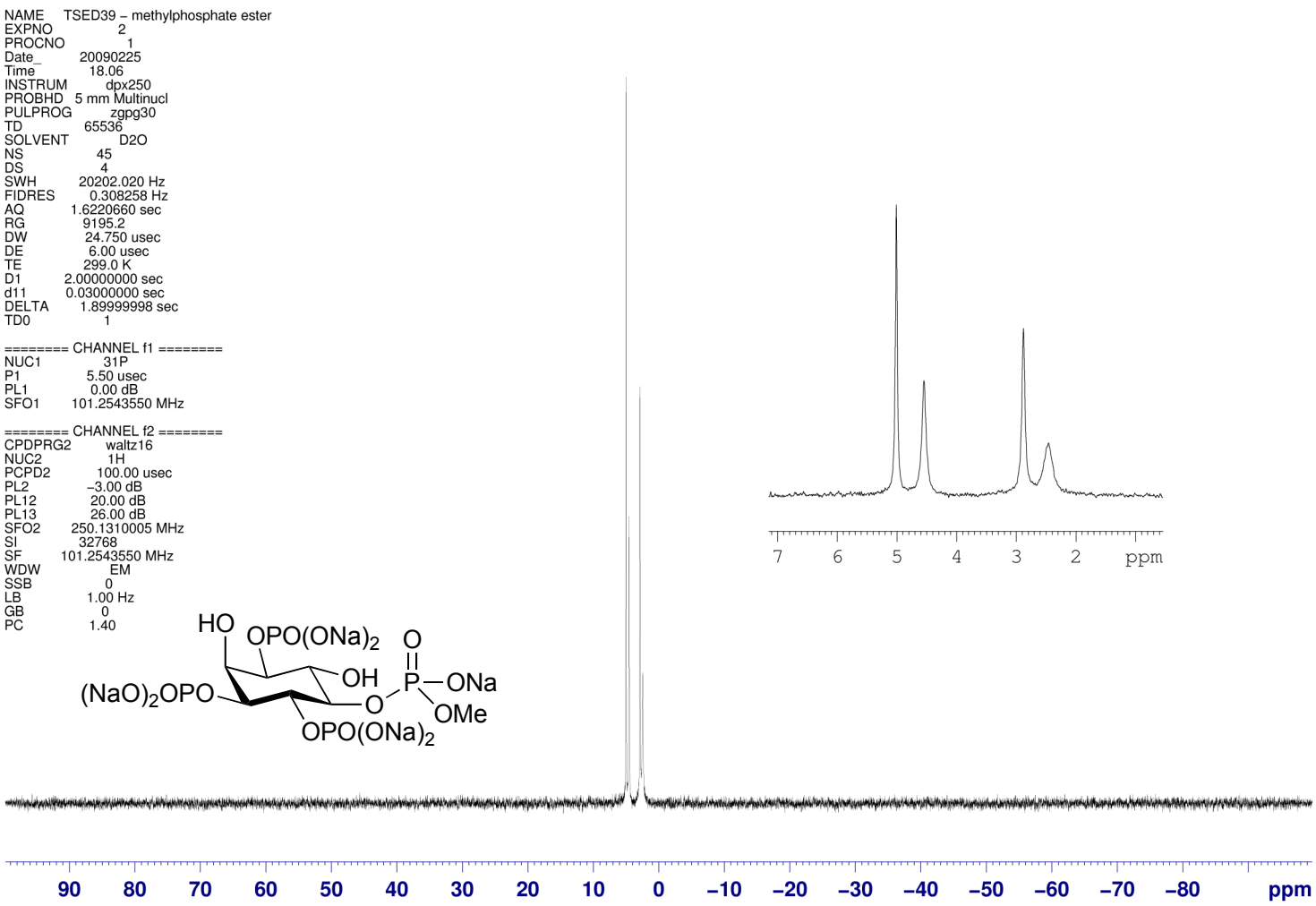
(-)-1D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-methylphosphate ester 157



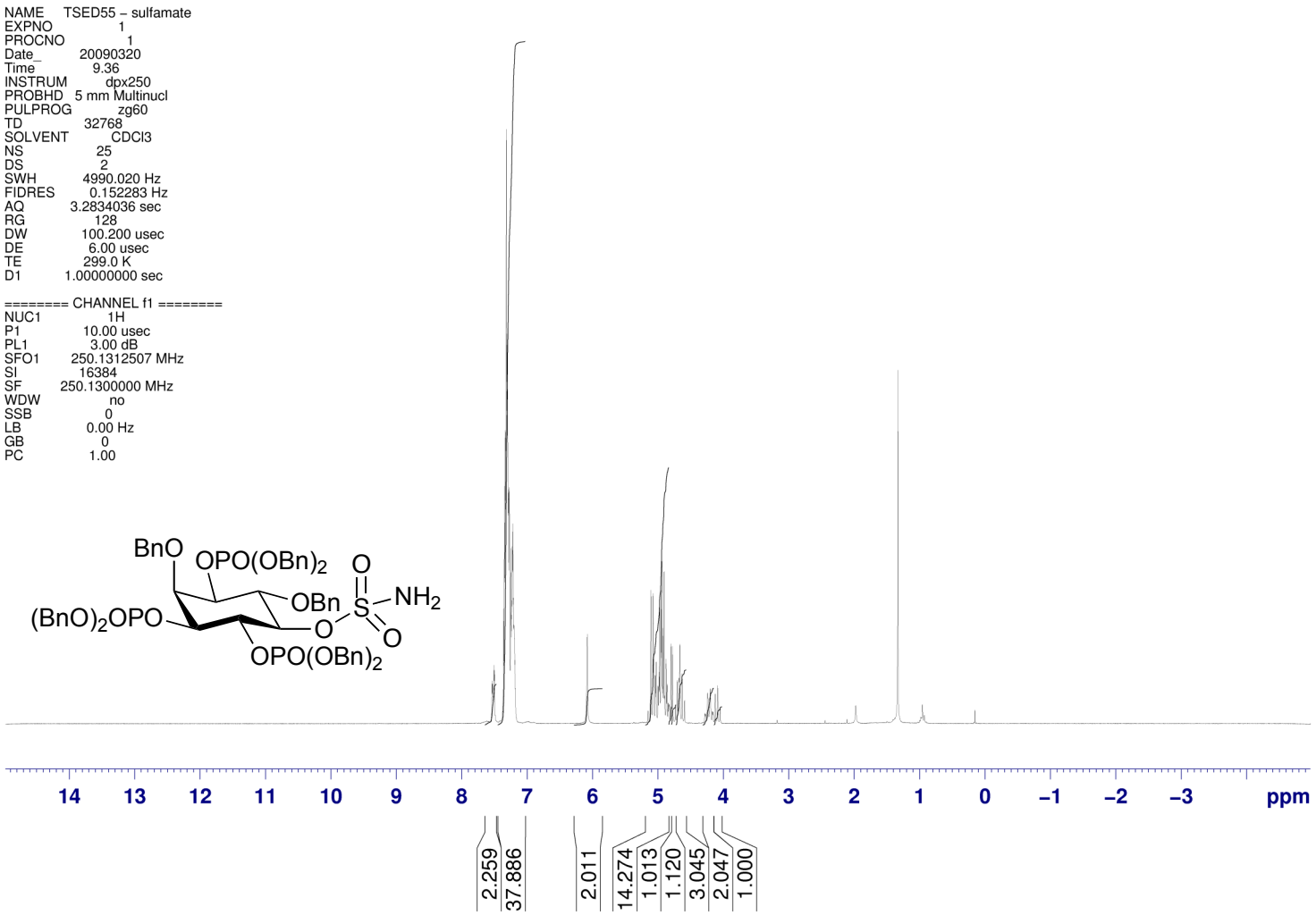
(-)-1D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-methylphosphate ester 157



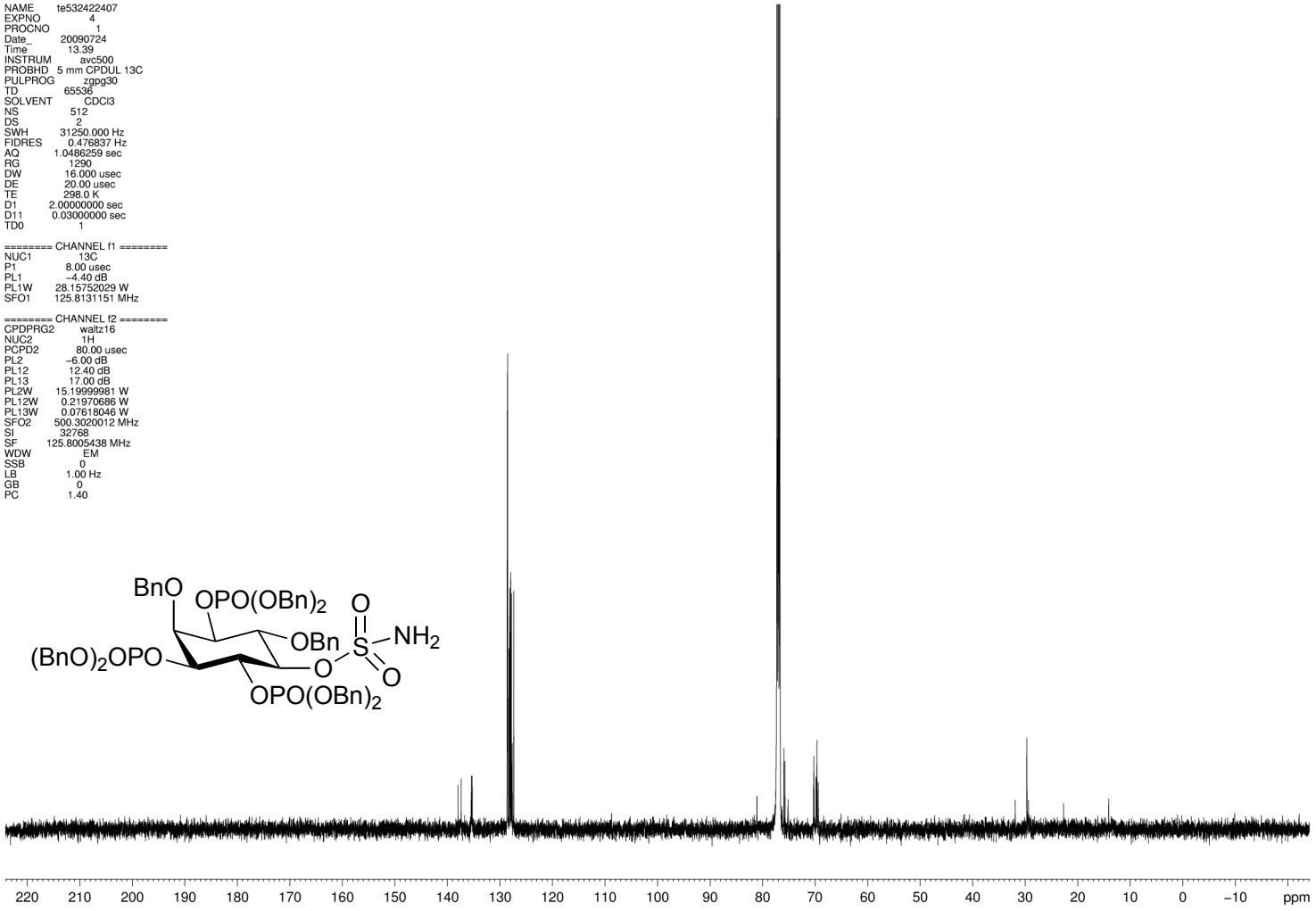
(-)-1D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-methylphosphate ester 157



(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 5-*O*-sulfamoly-1,3,4-tris(dibenzylphosphate) 159



(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 5-*O*-sulfamoly-1,3,4-tris(dibenzylphosphate) 159



(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 5-*O*-sulfamoly-1,3,4-tris(dibenzylphosphate) 159

NAME TSED55 – sulfamate
EXPNO 2
PROCNO 1
Date_ 20090320
Time 9.39
INSTRUM dpx250
PROBHD 5 mm Multinucl
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 20
DS 4
SWH 20202.020 Hz
FIDRES 0.308258 Hz
AQ 1.6220660 sec
RG 9195.2
DW 24.750 usec
DE 6.00 usec
TE 299.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1

======== CHANNEL f1 ========
NUC1 31P
P1 5.50 usec
PL1 0.00 dB
SFO1 101.2543550 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 –3.00 dB
PL12 20.00 dB
PL13 26.00 dB
SFO2 250.1310005 MHz
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SF 101.2543550 MHz
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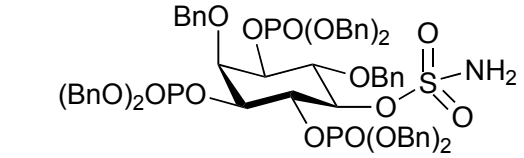
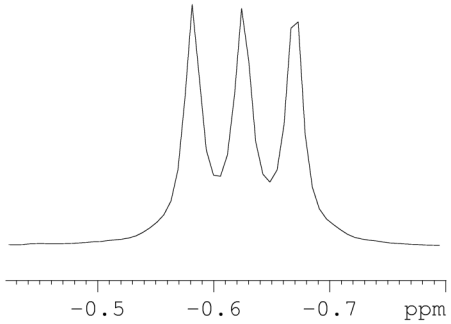
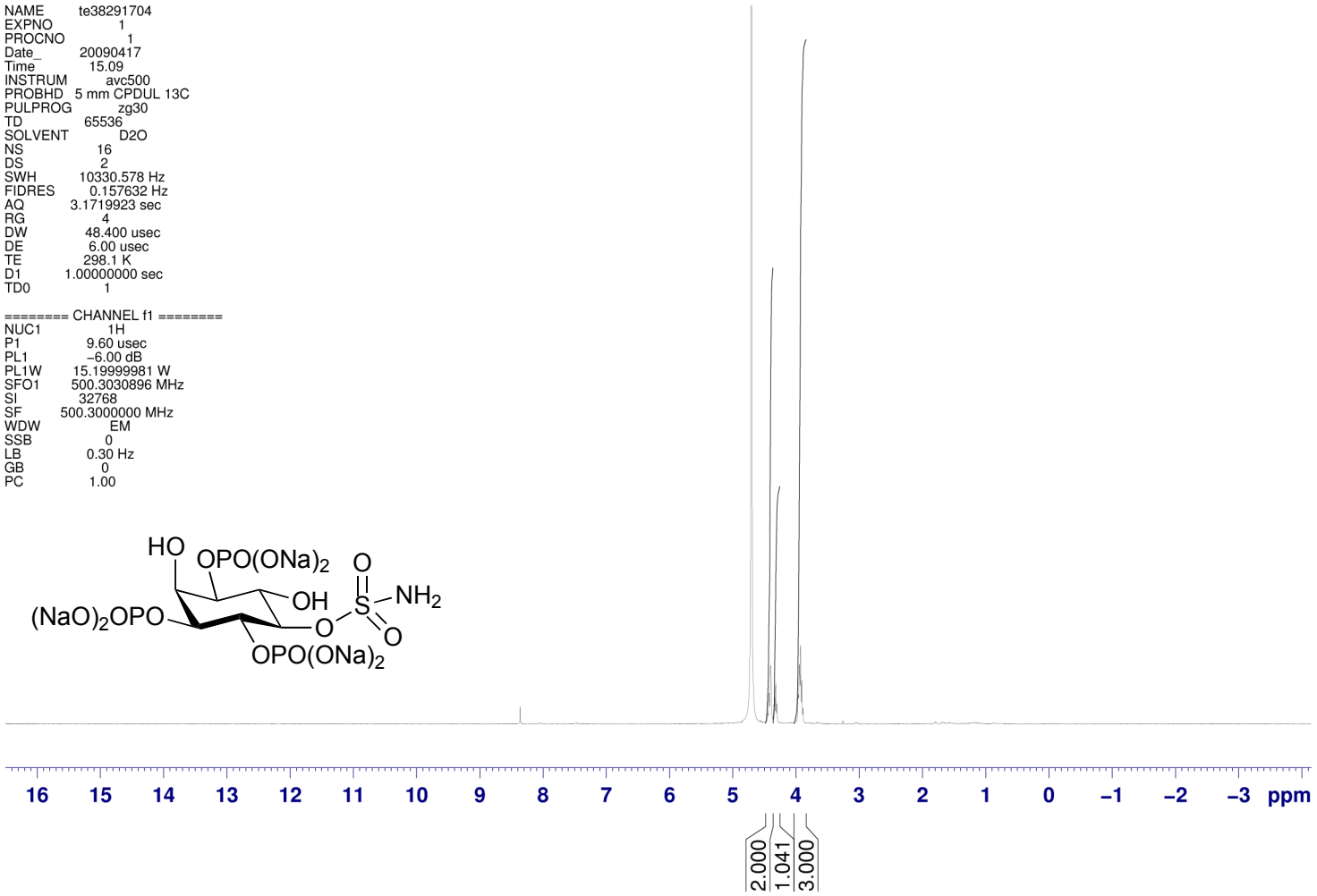
SSB 0
LB 1.00 Hz
GB 0
PC 1.40





(+)-1D-*myo*-Inositol-1,3,4-trisphosphate-5-*O*-sulfamate 160



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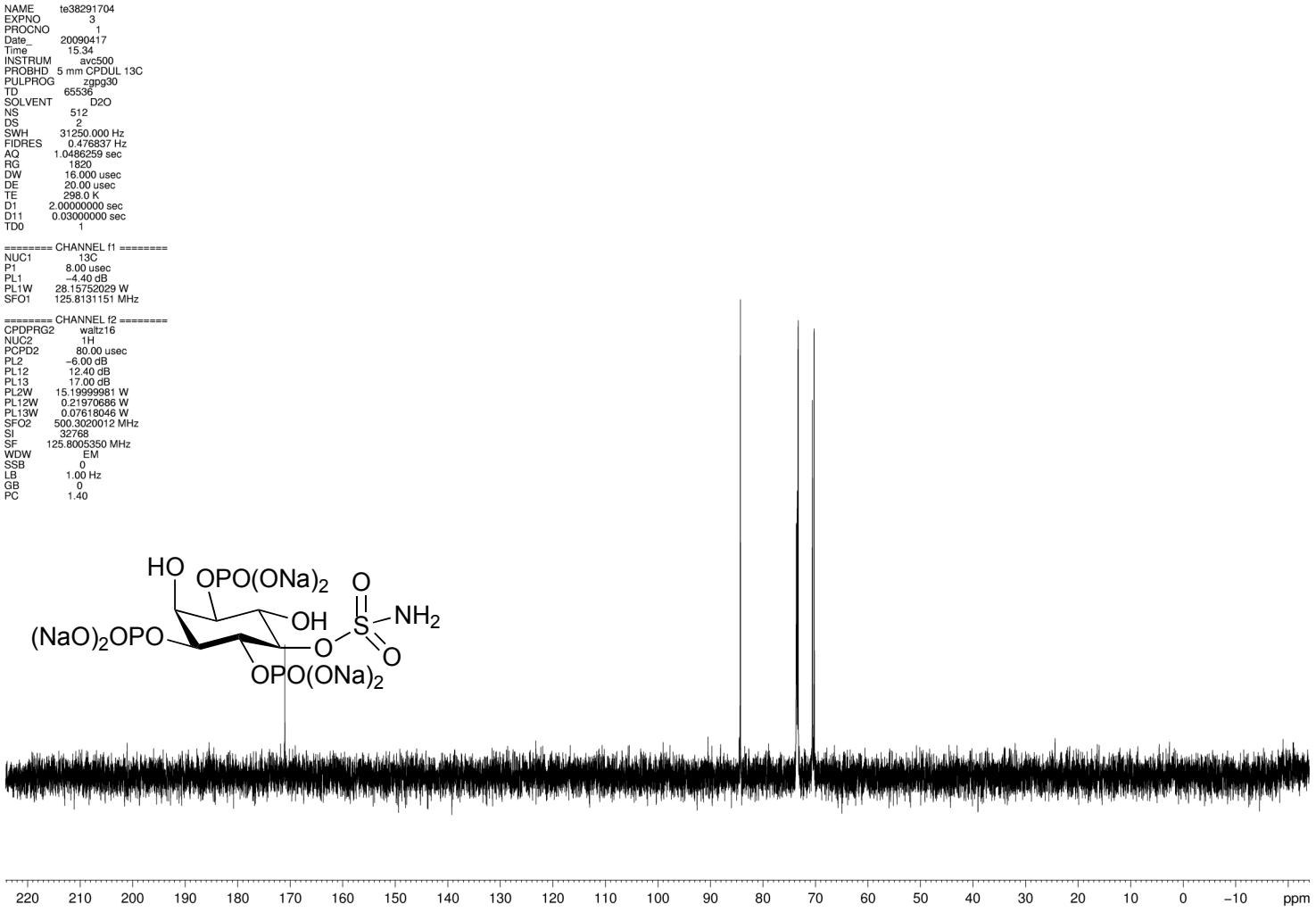

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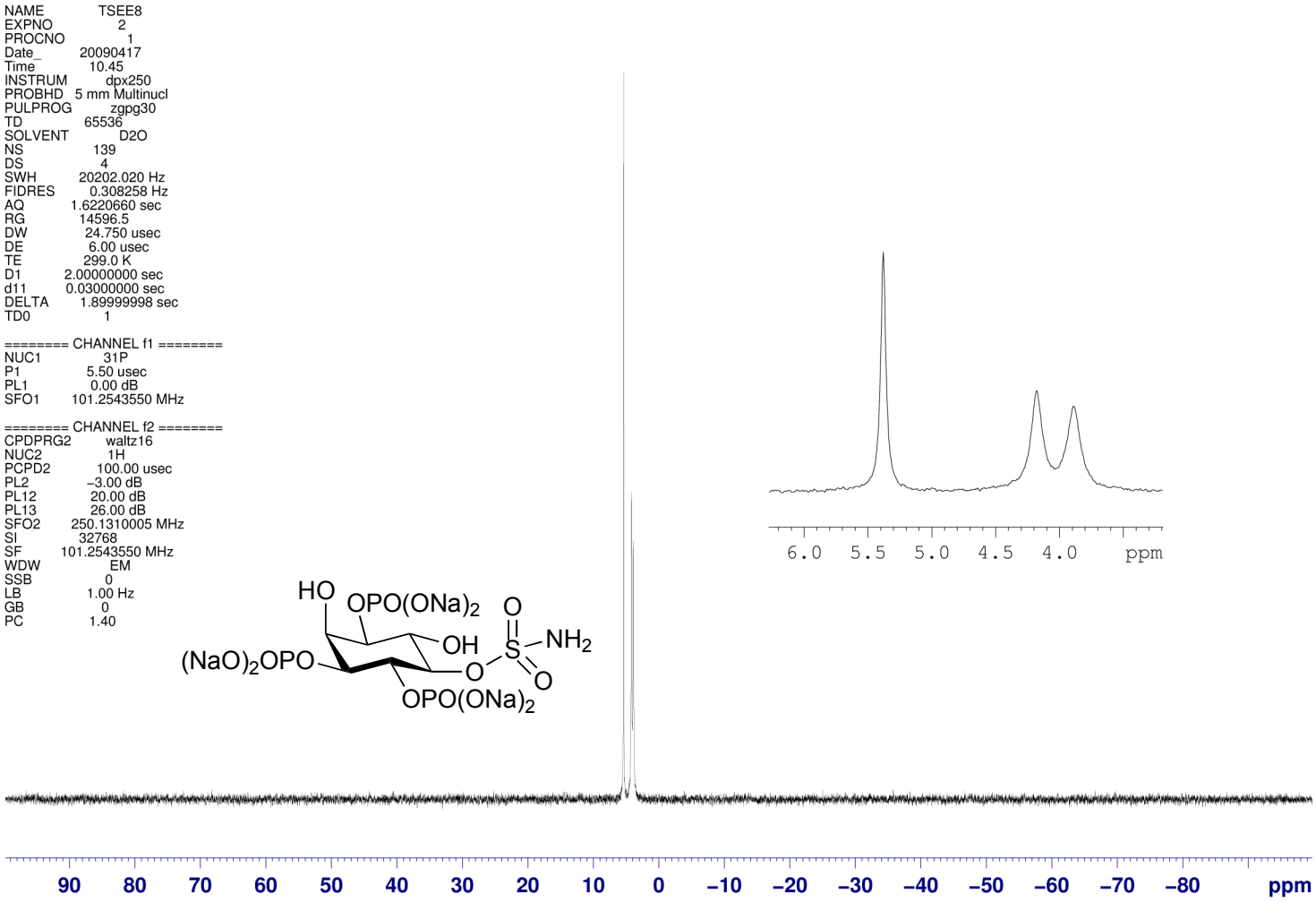
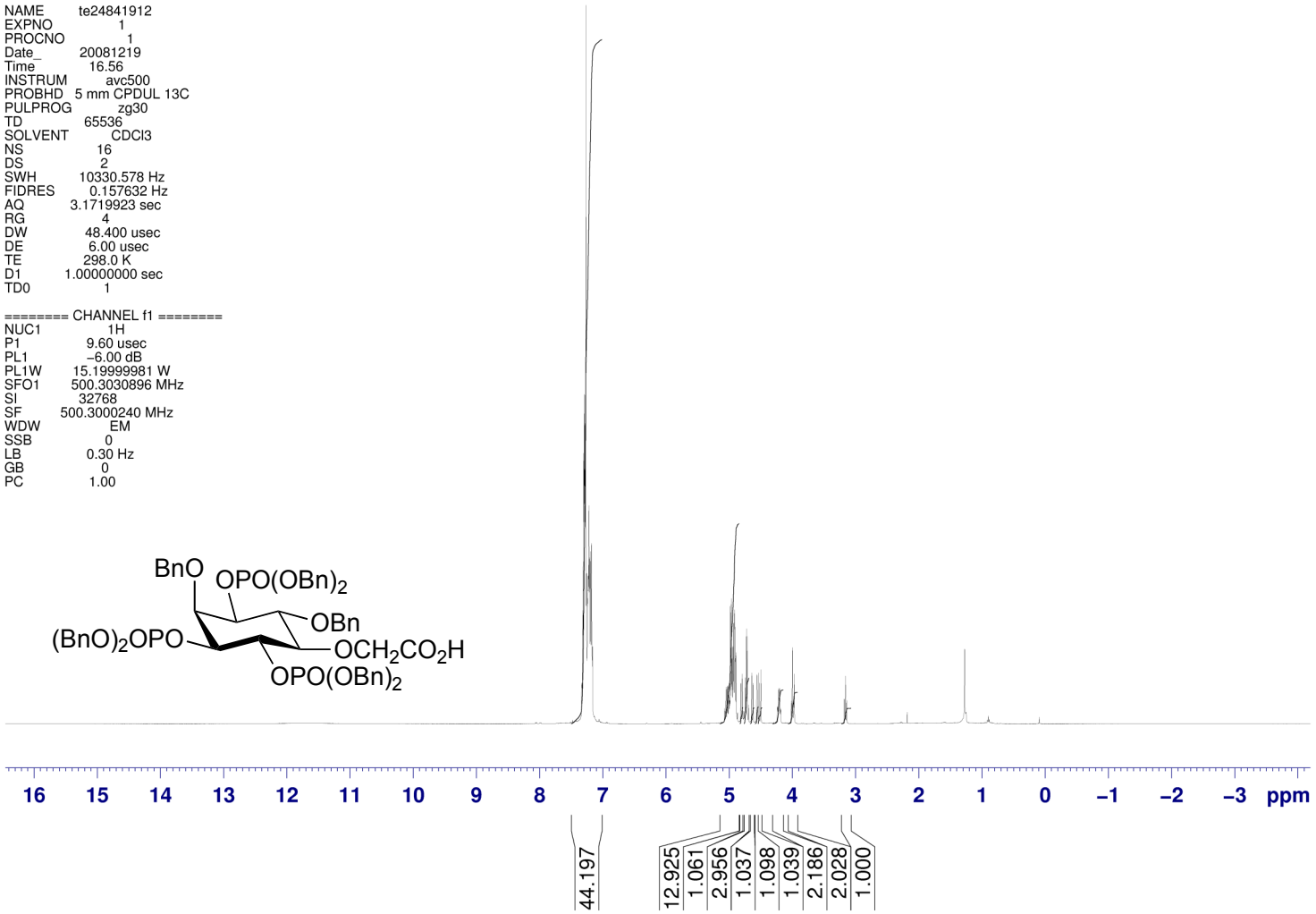


(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate)-5-*O*-acetic acid 172



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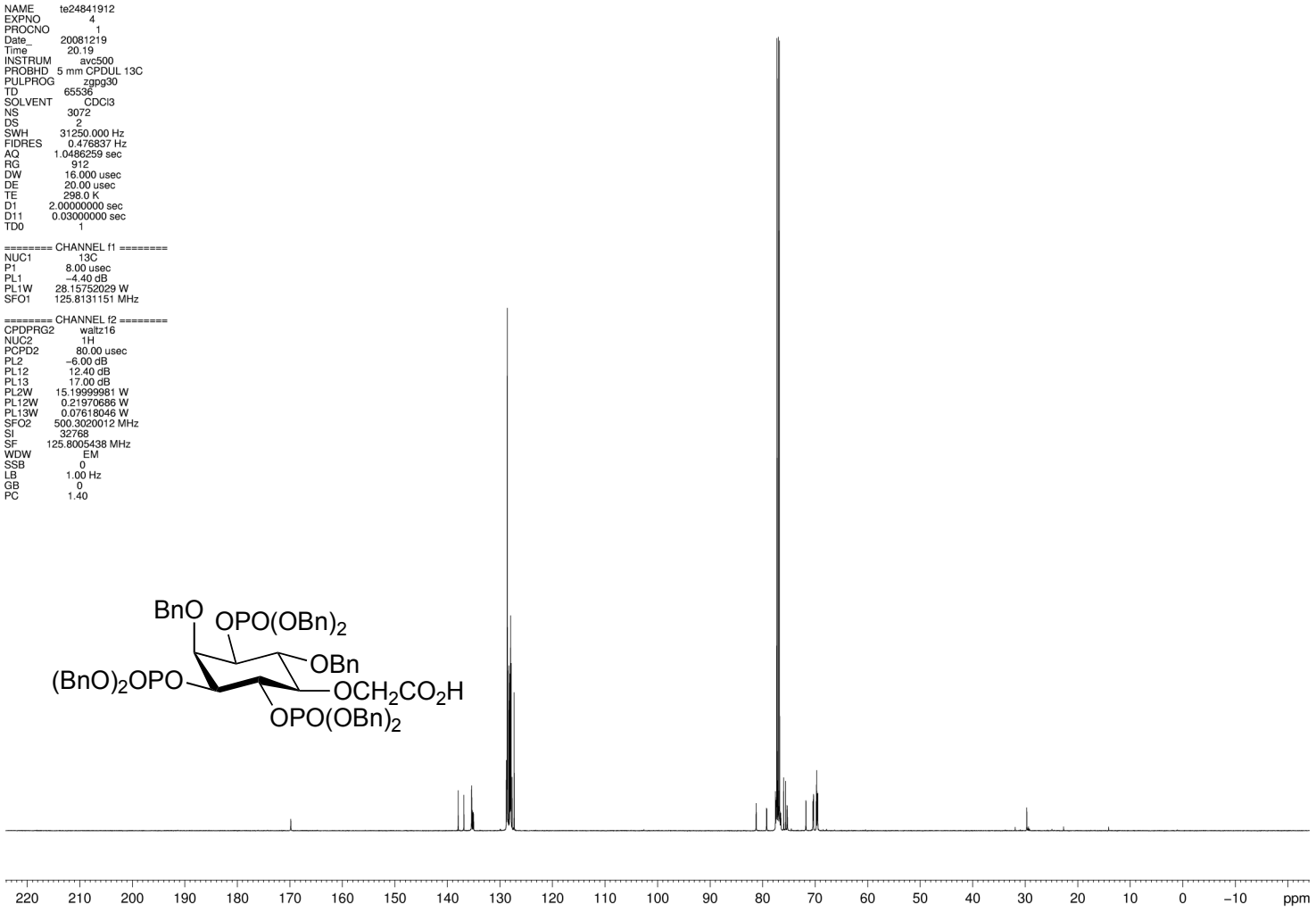
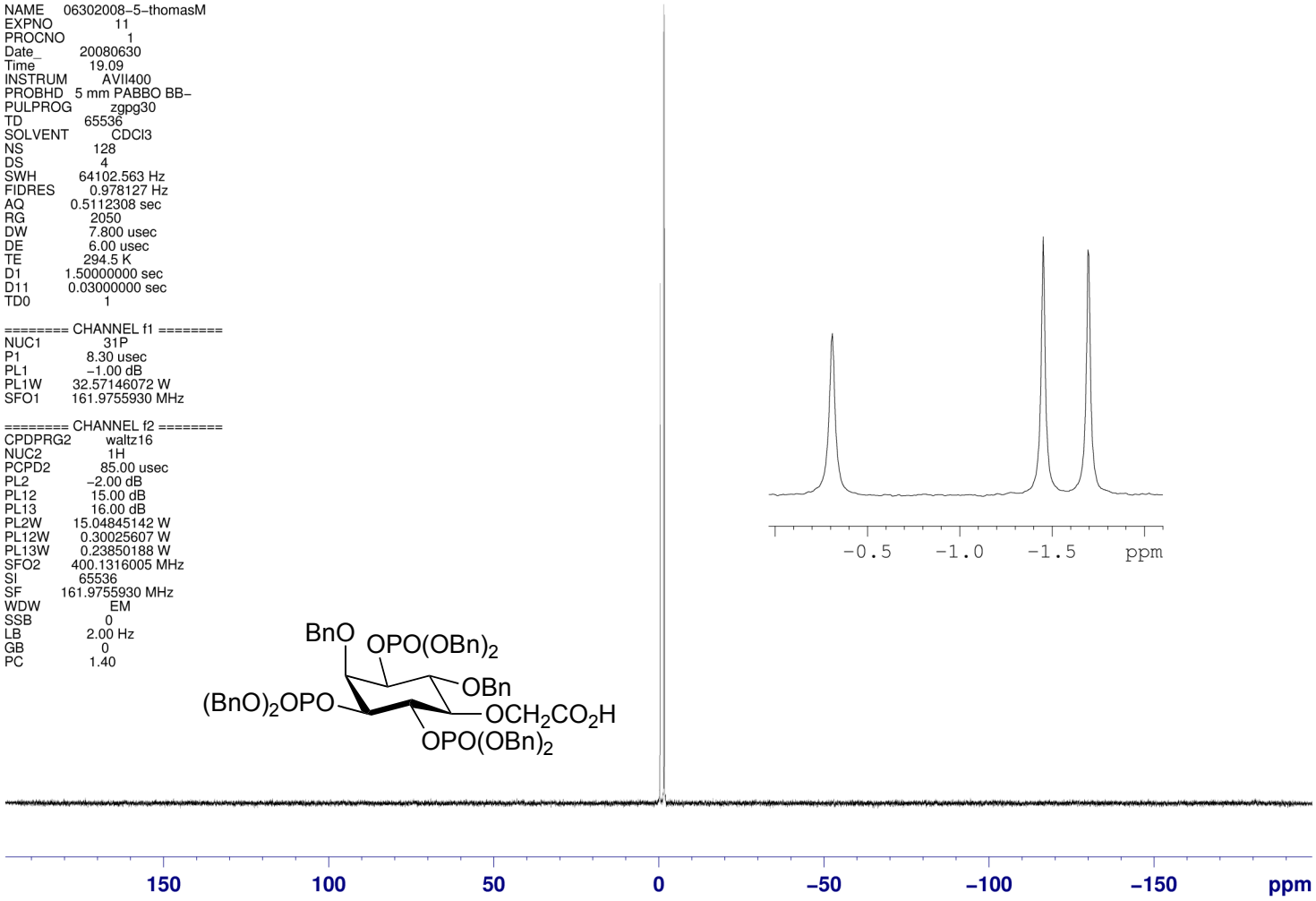


(+)-1D-2,6-bis-*O*-Benzyl-*myo*-inositol 1,3,4-tris(dibenzylphosphate)-5-*O*-acetic acid 172



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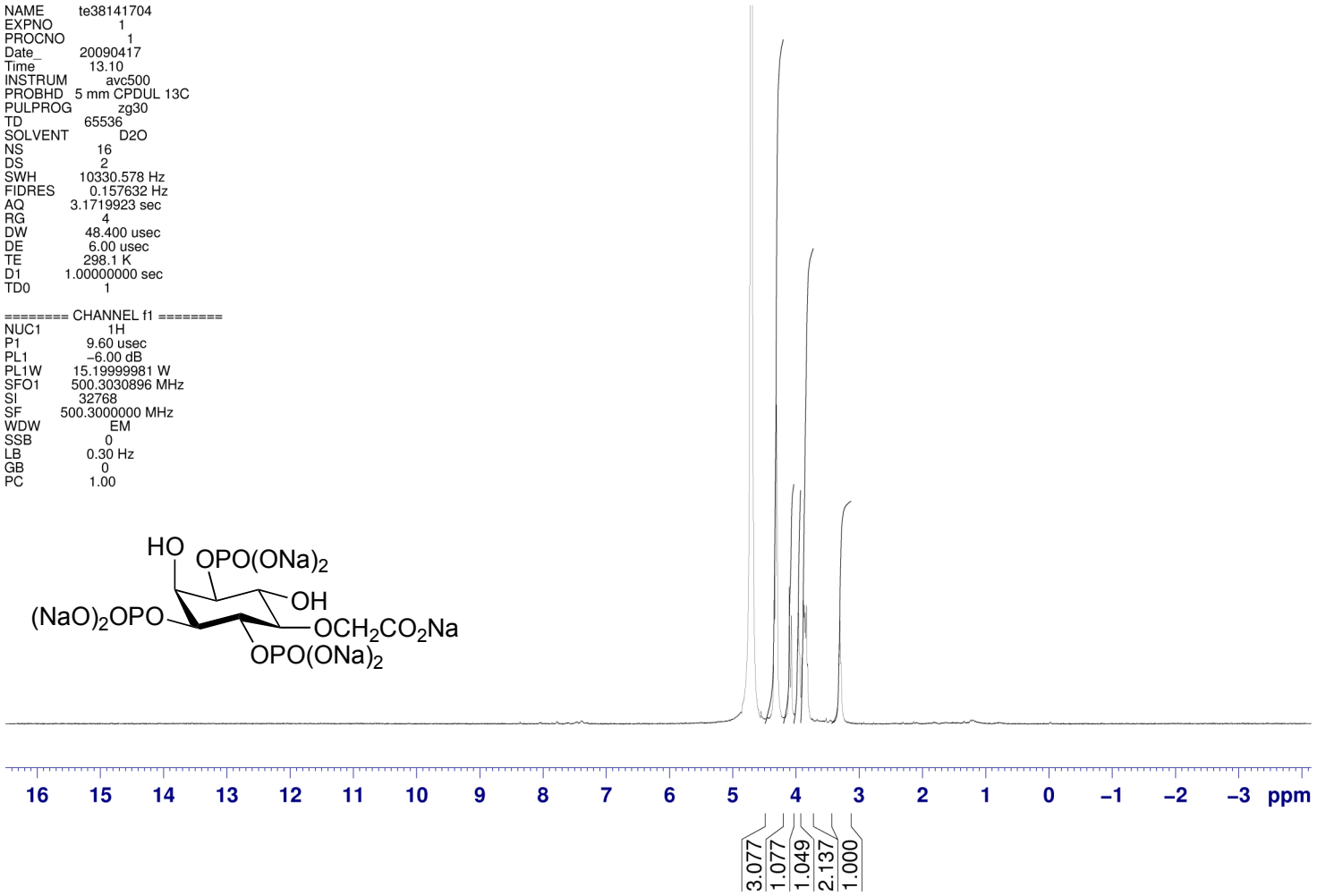
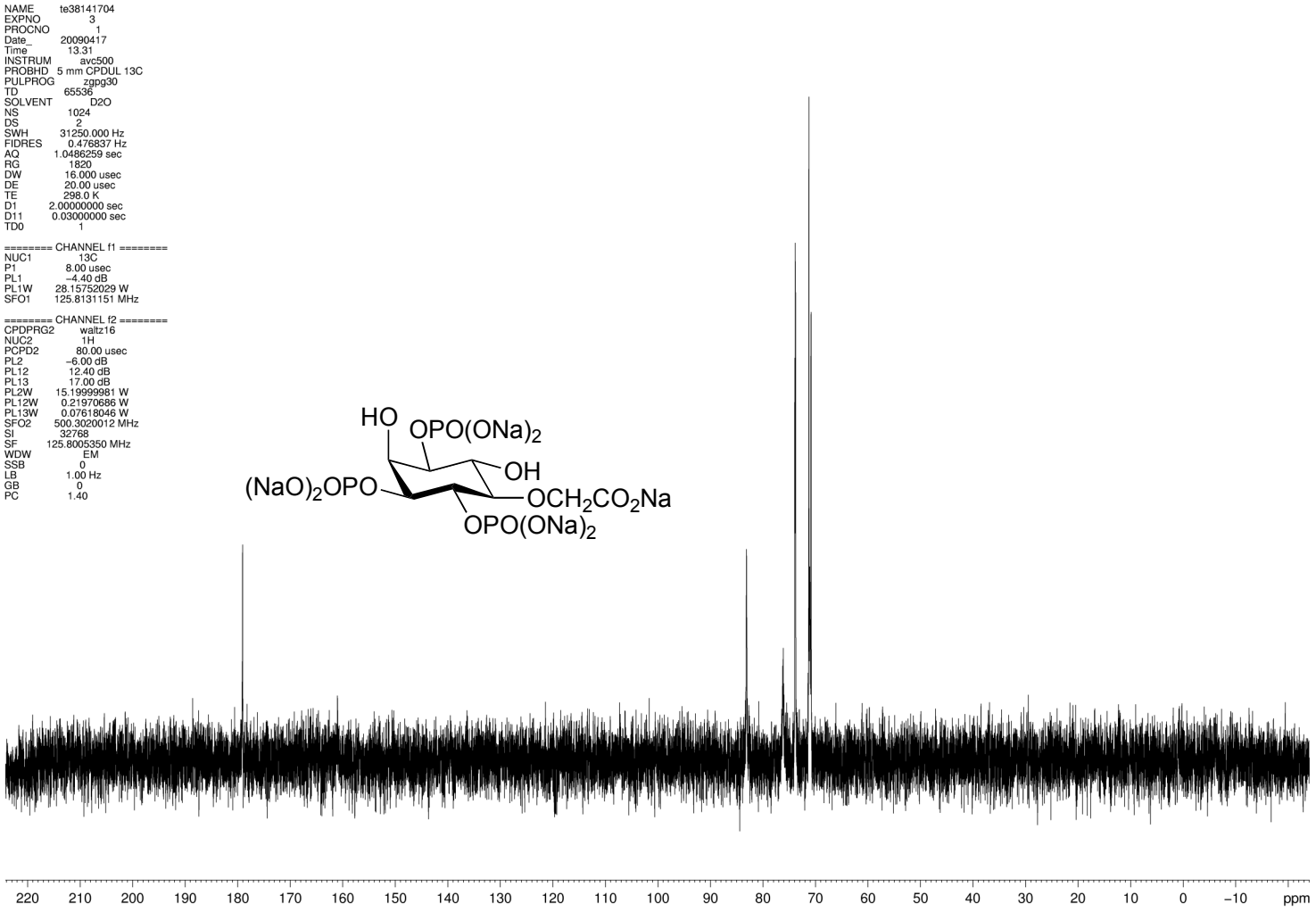
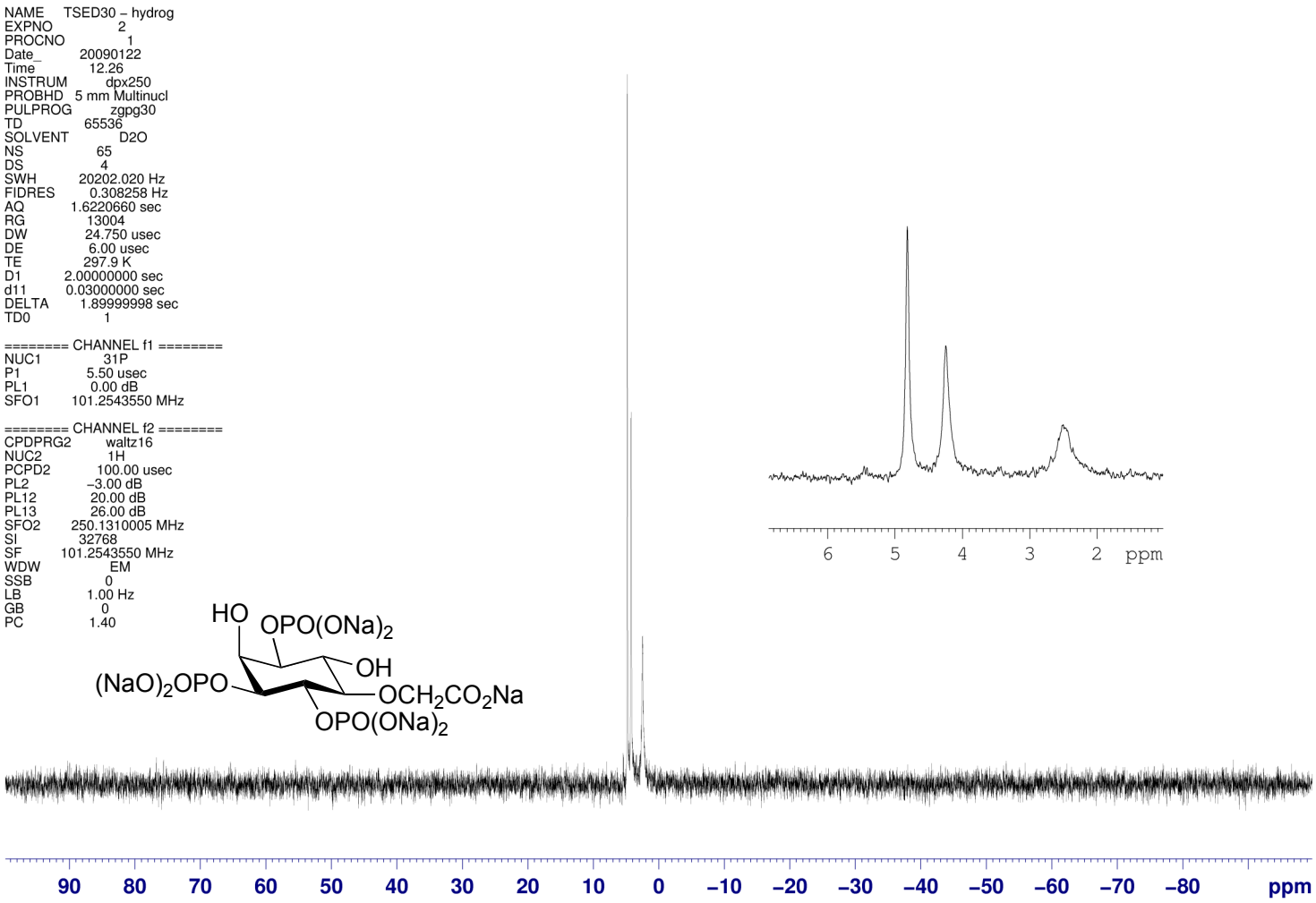


(-)-1D-*myo*-Inositol 1,3,4-trisphosphate-5-*O*-acetic acid 173



(-)-1D-*myo*-Inositol 1,3,4-trisphosphate-5-*O*-acetic acid 173



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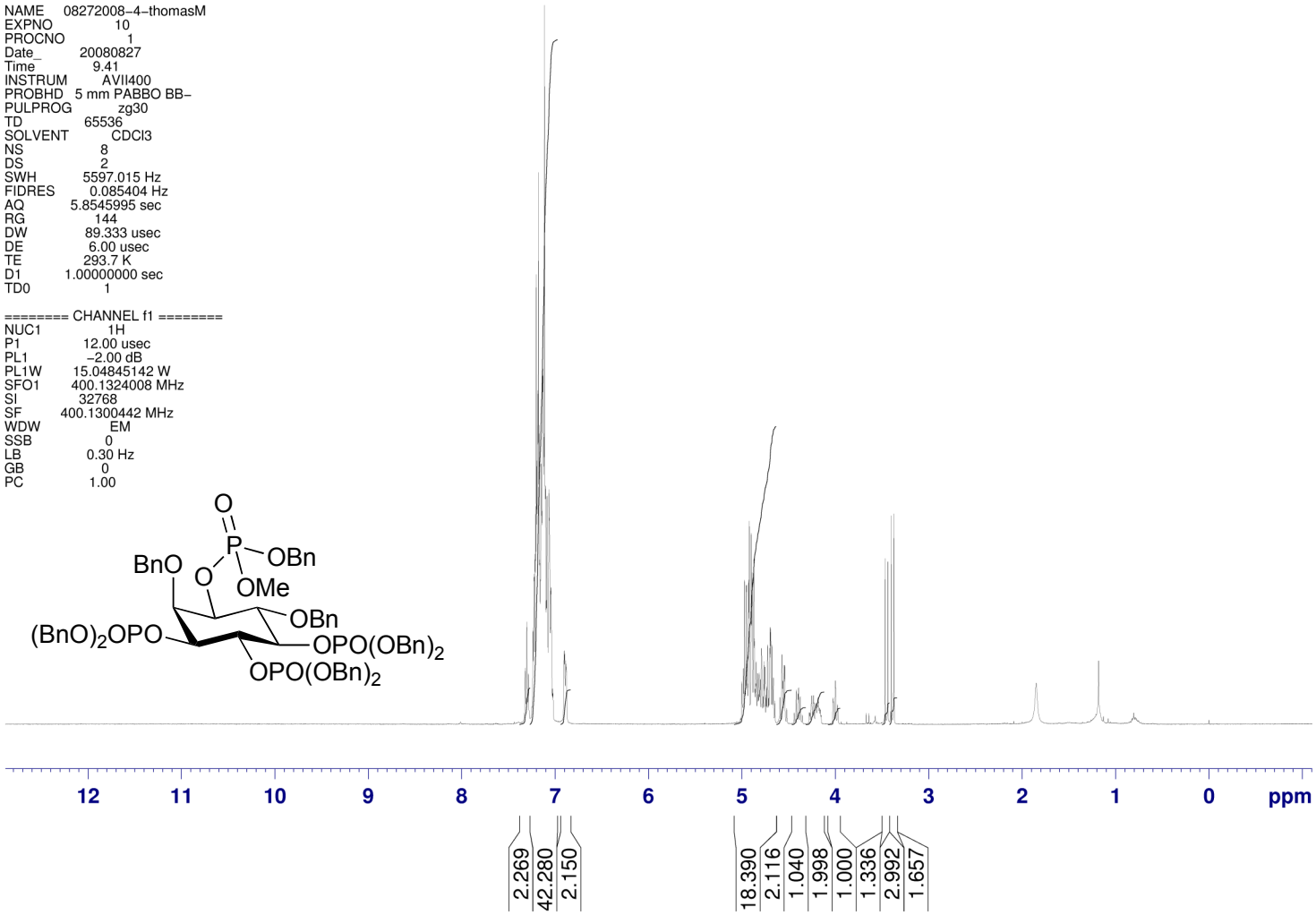
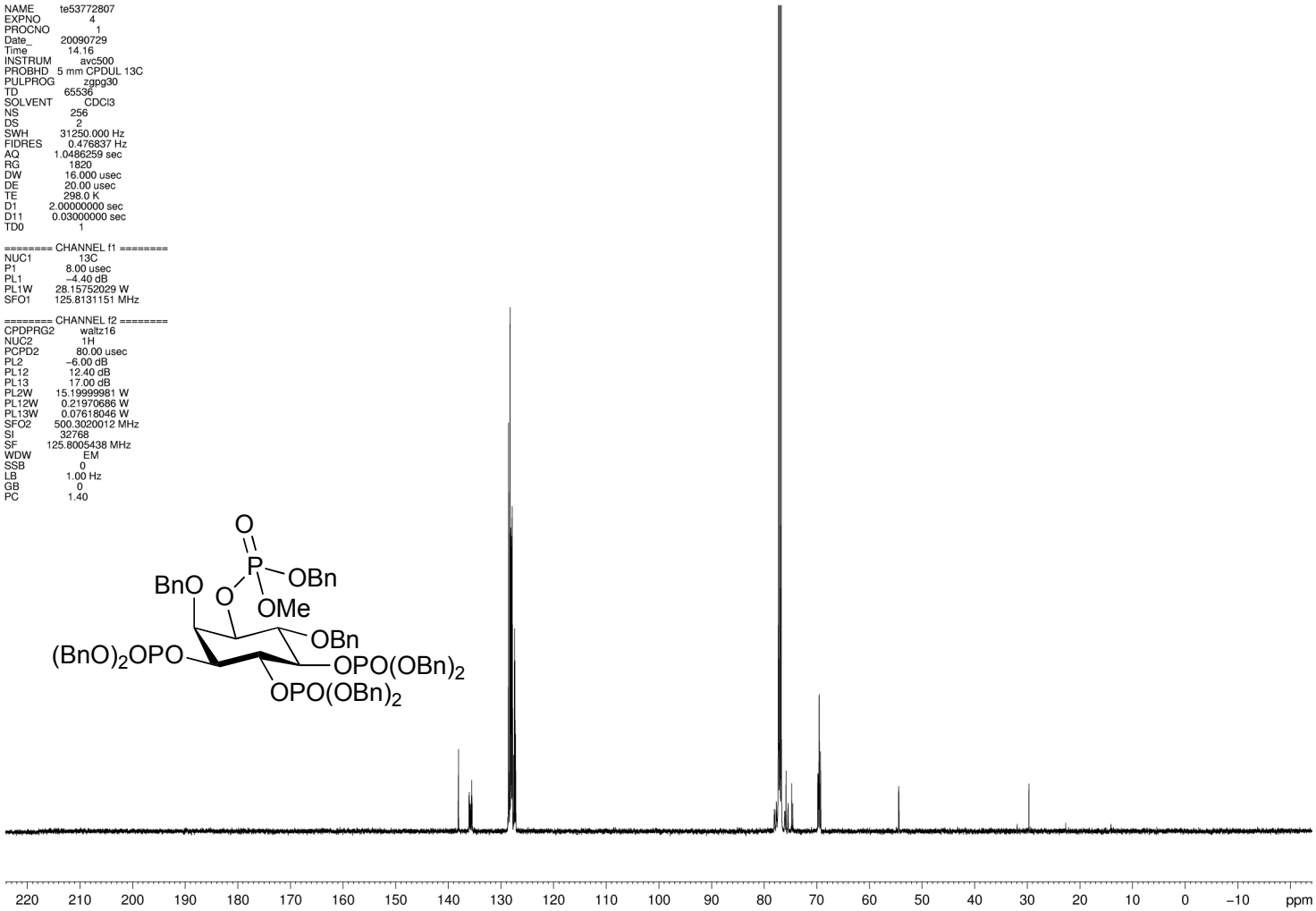
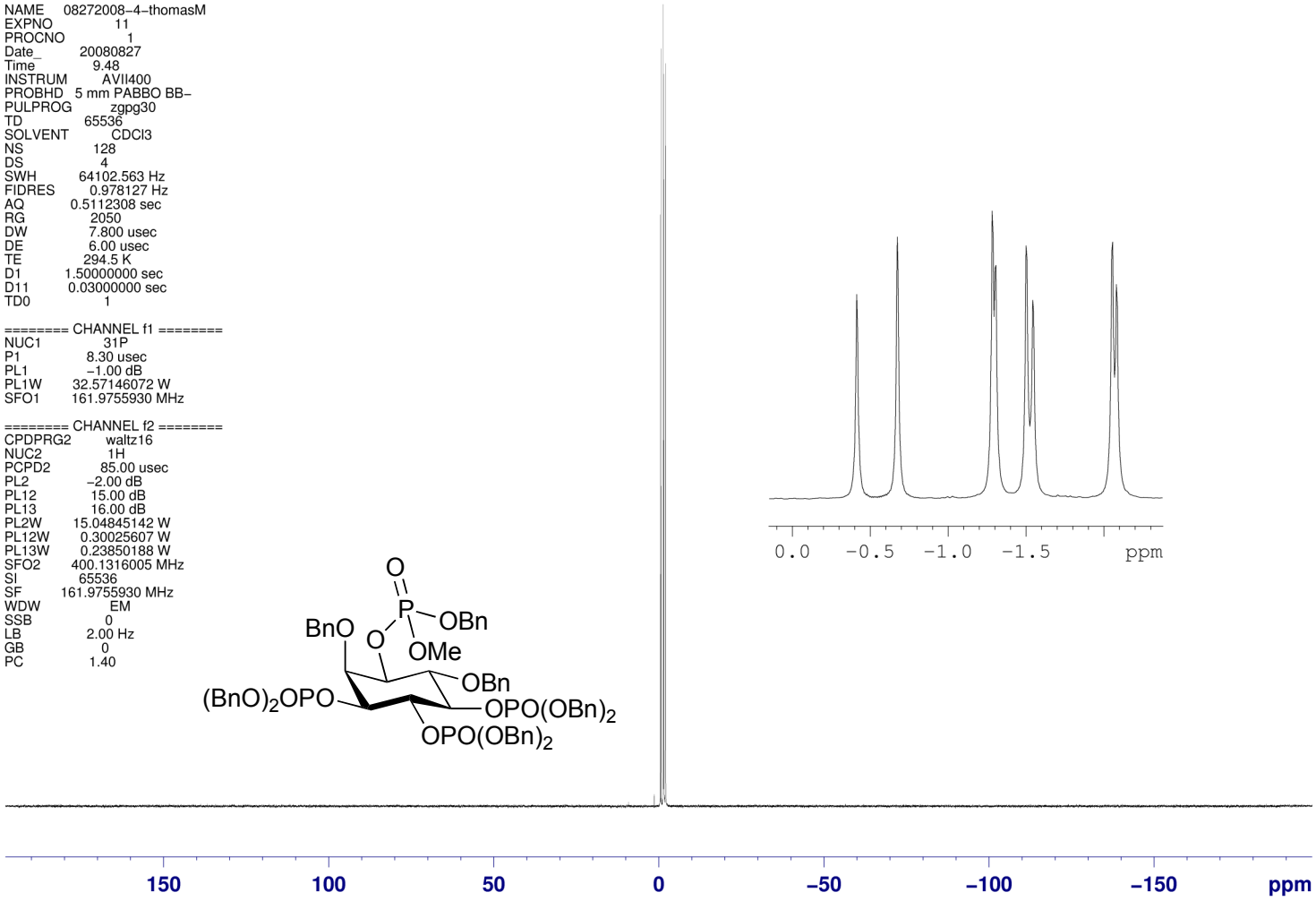


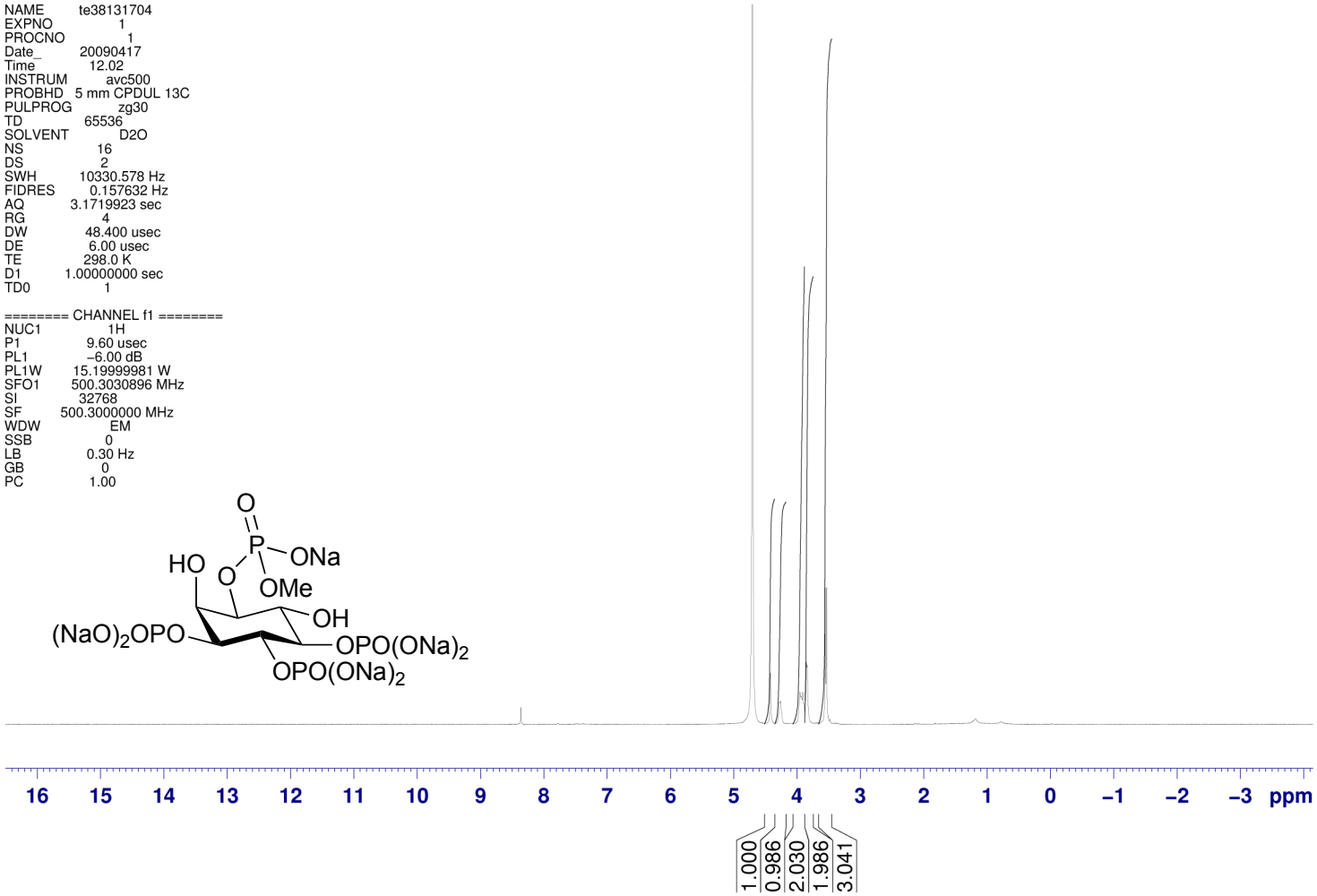
(-)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1-*O*-benzyl-*O*-methylphosphate-3,4,5-tris(dibenzylphosphate) 199



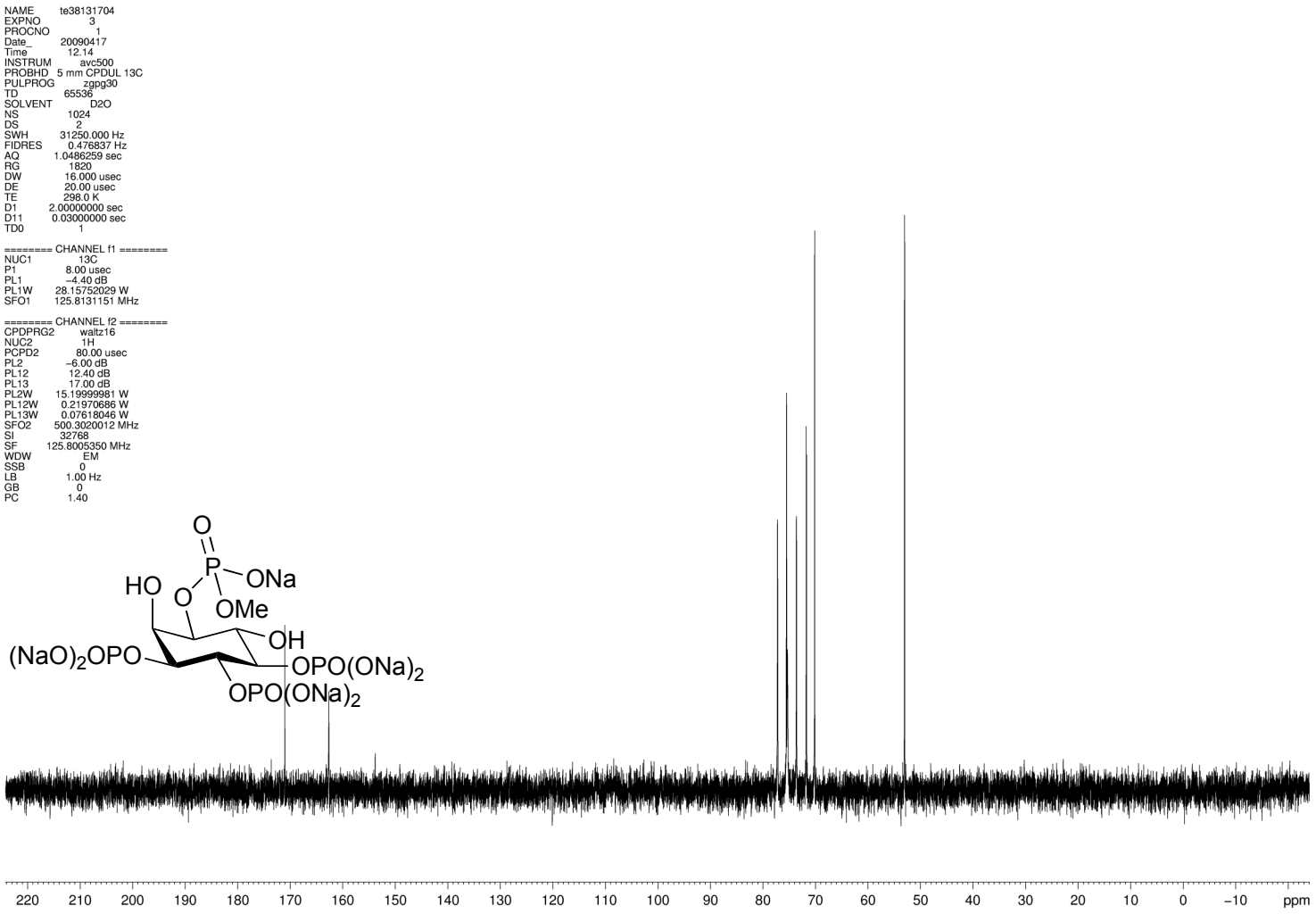
(-)-1D-2,6-bis-*O*-benzyl-*myo*-inositol 1-*O*-benzyl-*O*-methylphosphate-3,4,5-tris(dibenzylphosphate) 199



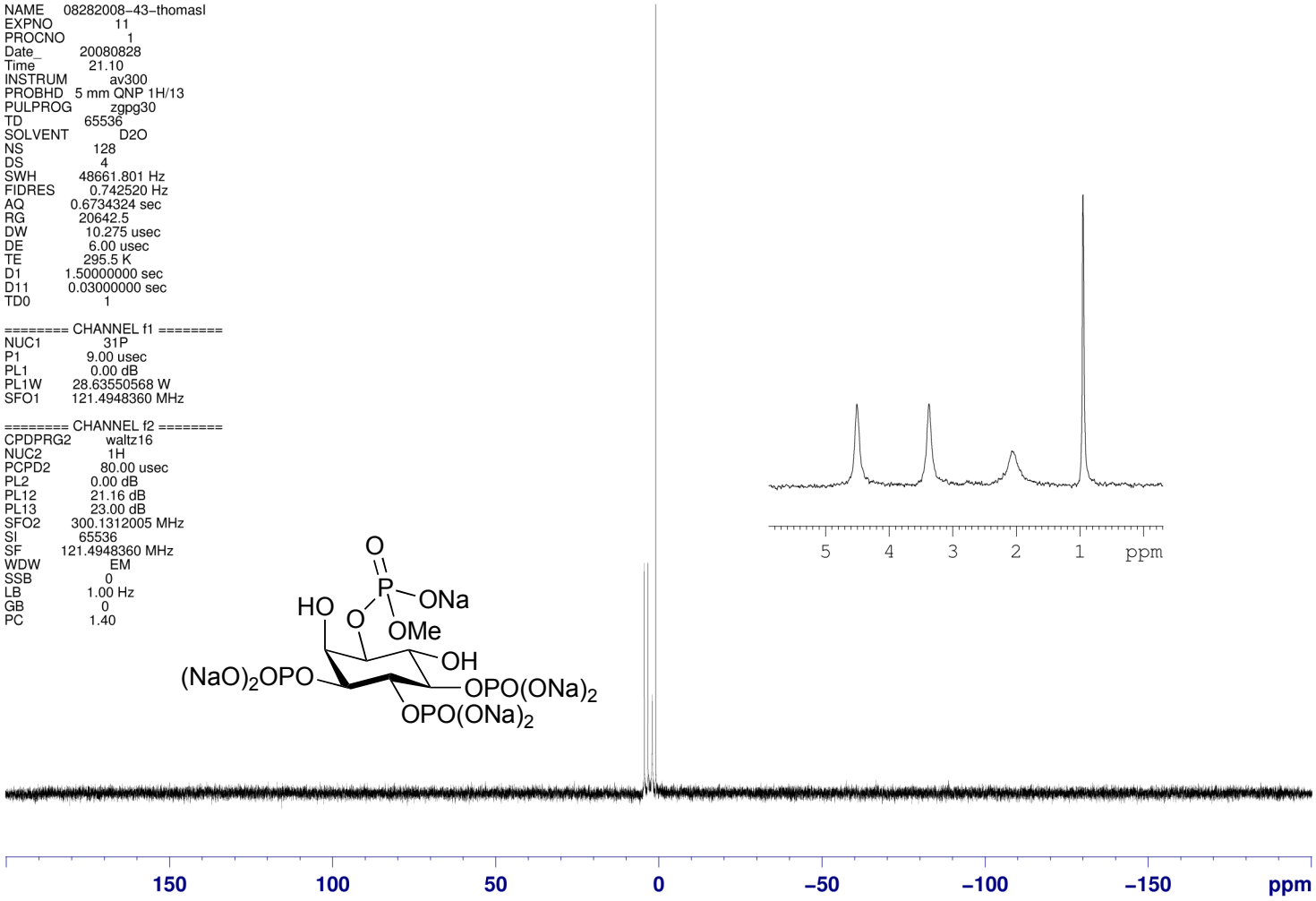
(-)-1D-*myo*-Inositol 1-*O*-methylphosphate-3,4,5-tris(phosphate) 198



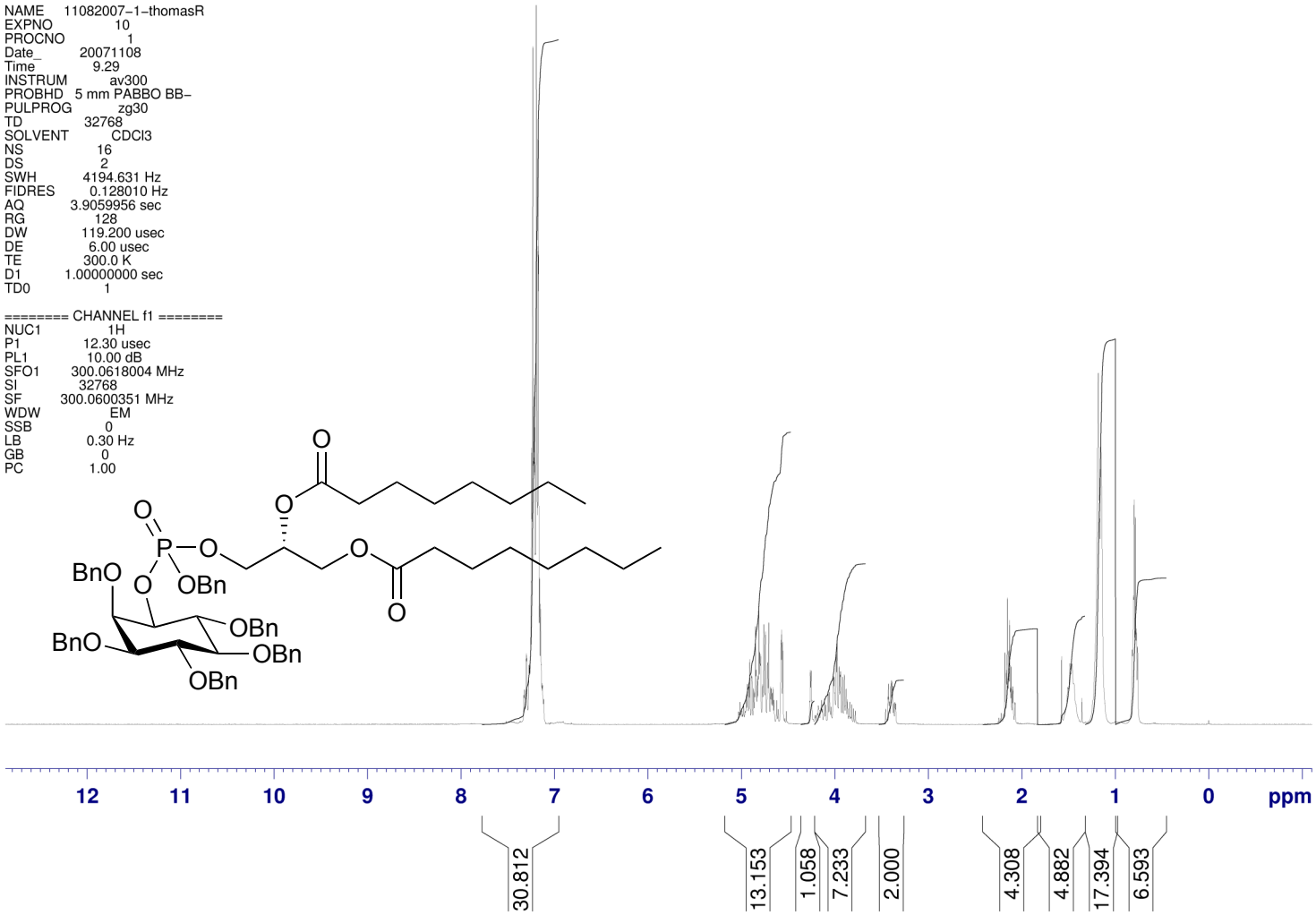
(-)-1D-*myo*-Inositol 1-*O*-methylphosphate-3,4,5-tris(phosphate) 198



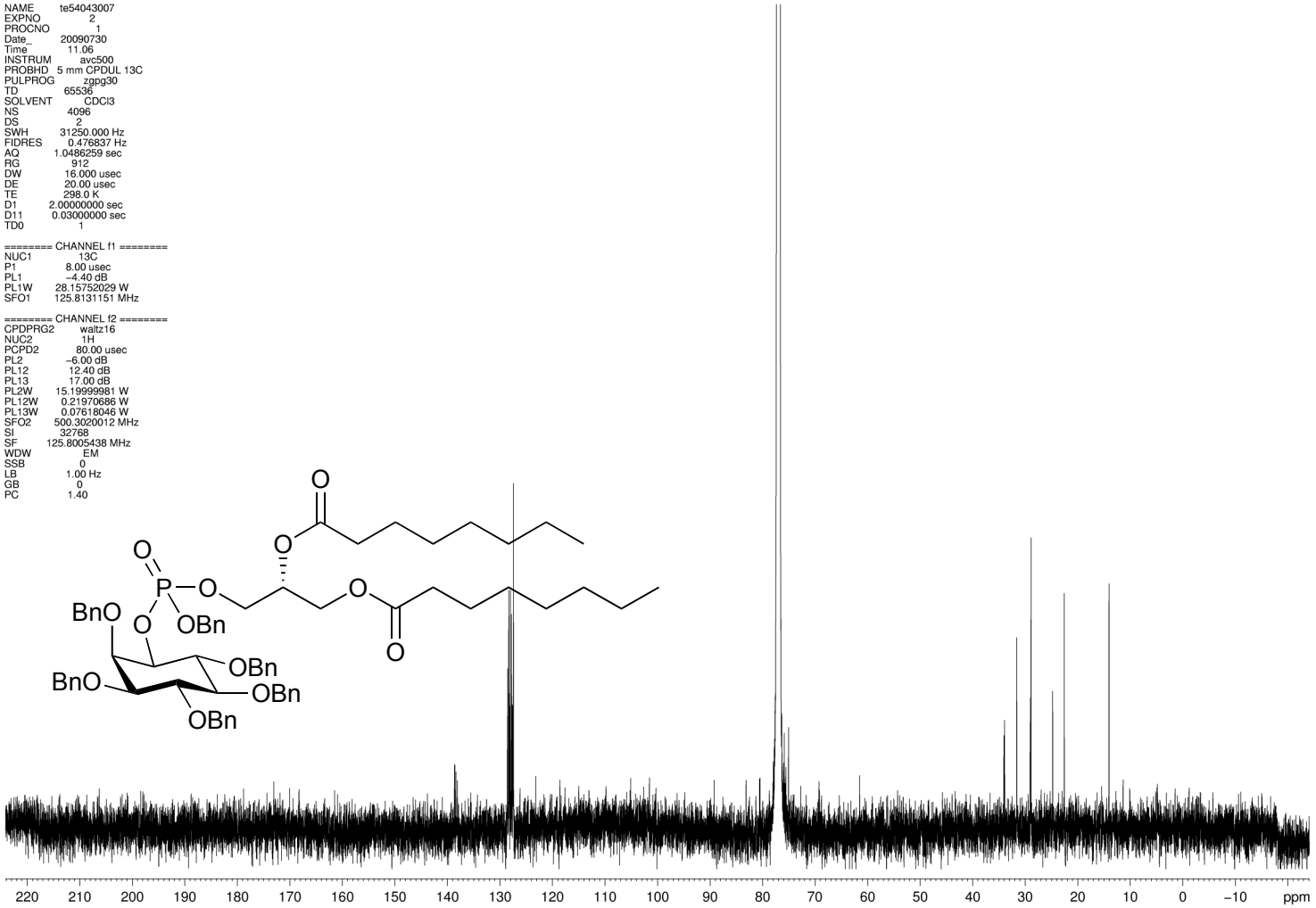
(-)-1D-*myo*-Inositol 1-*O*-methylphosphate-3,4,5-tris(phosphate) 198



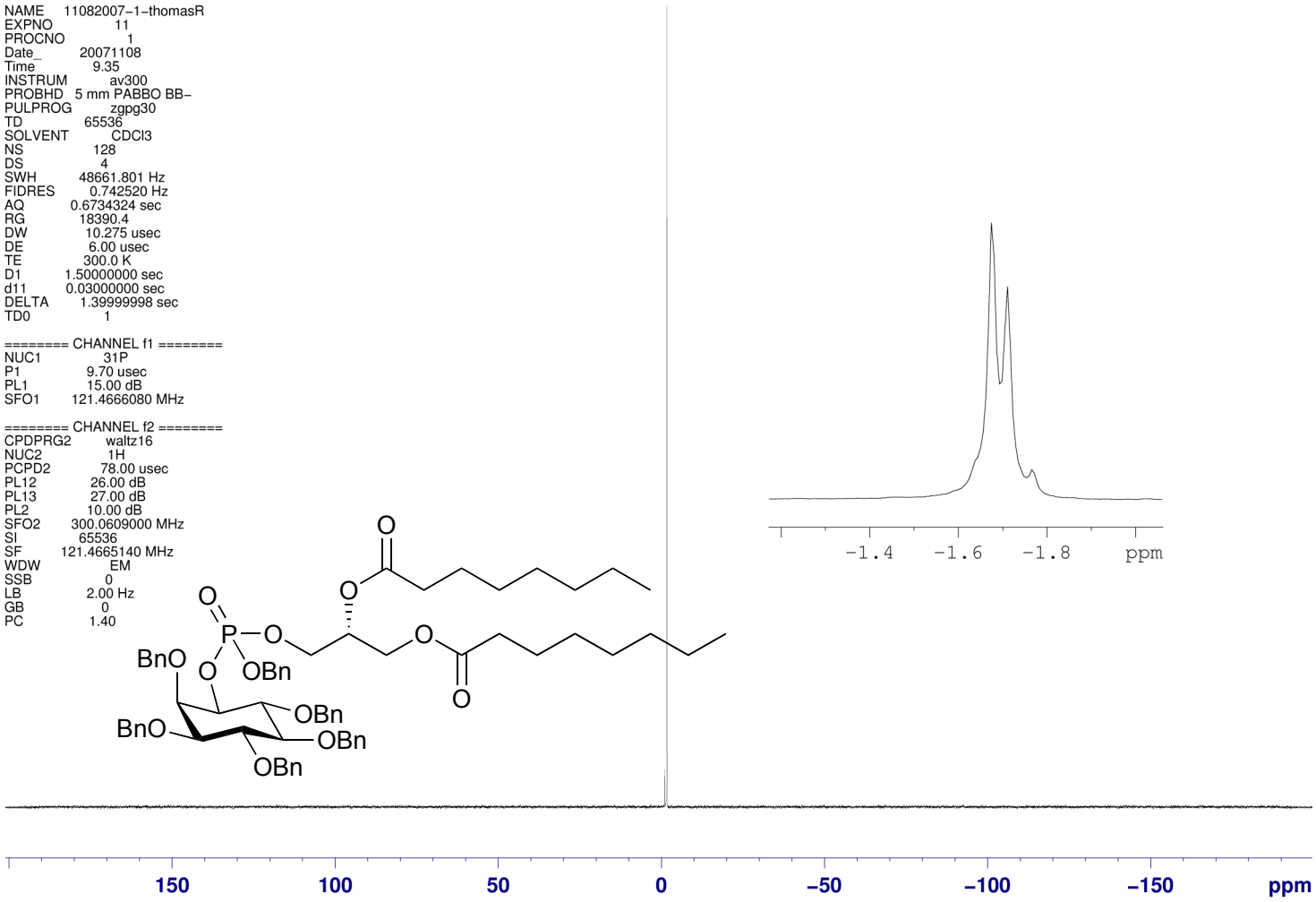
(+)-1D-1-(1,2-Dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (+)-204



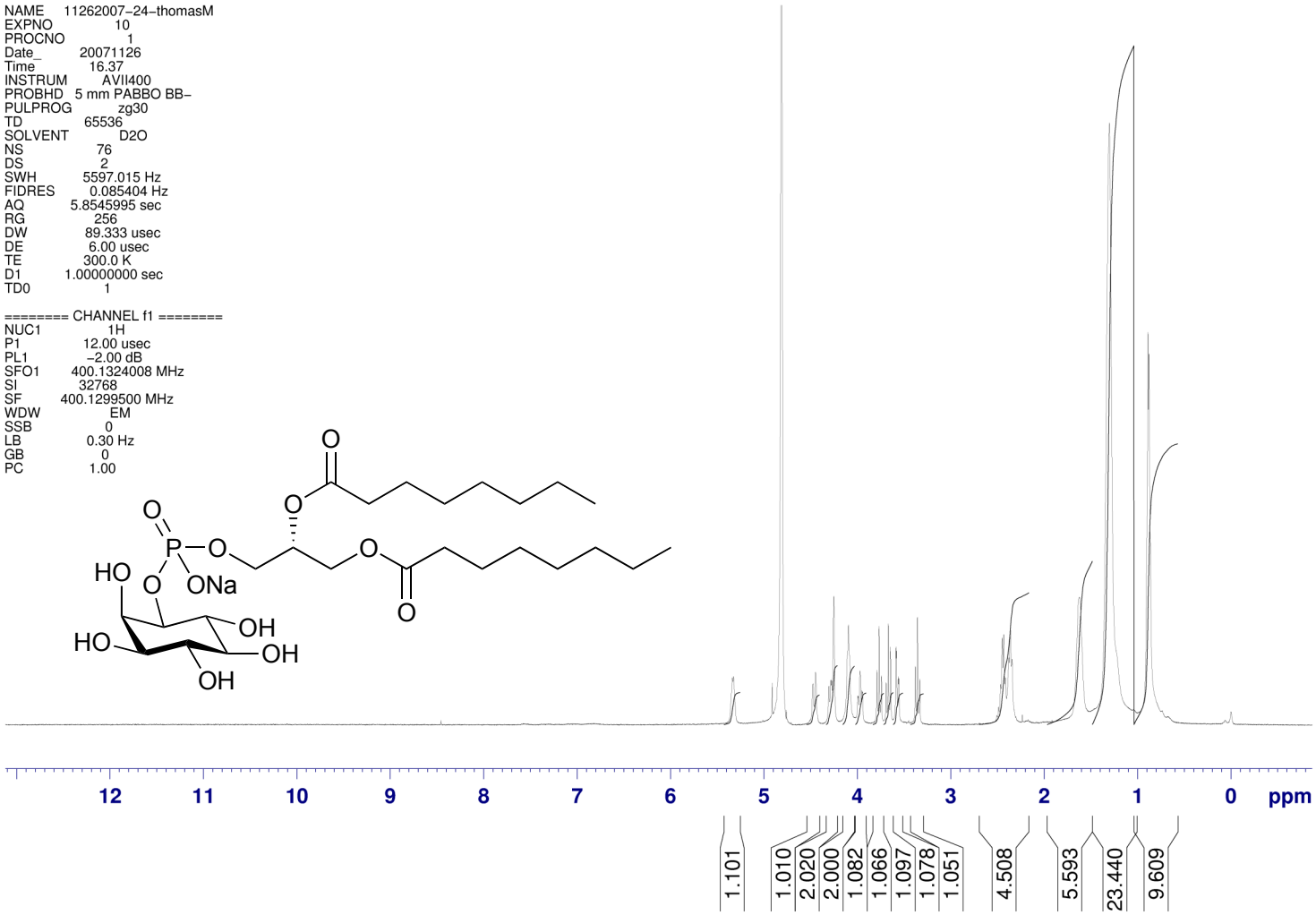
(+)-1D-1-(1,2-Dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (+)-204



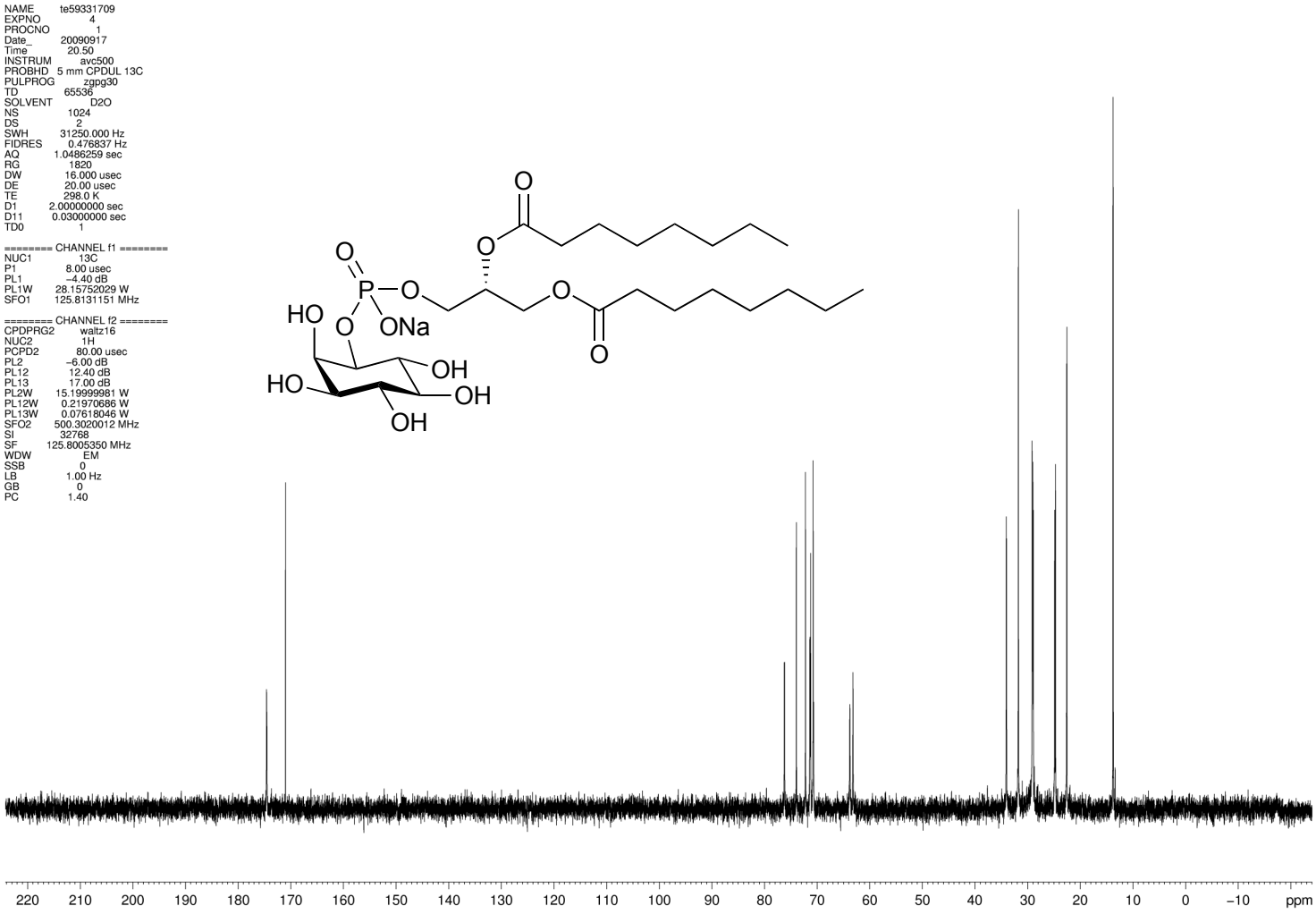
(+)-1D-1-(1,2-Dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (+)-204



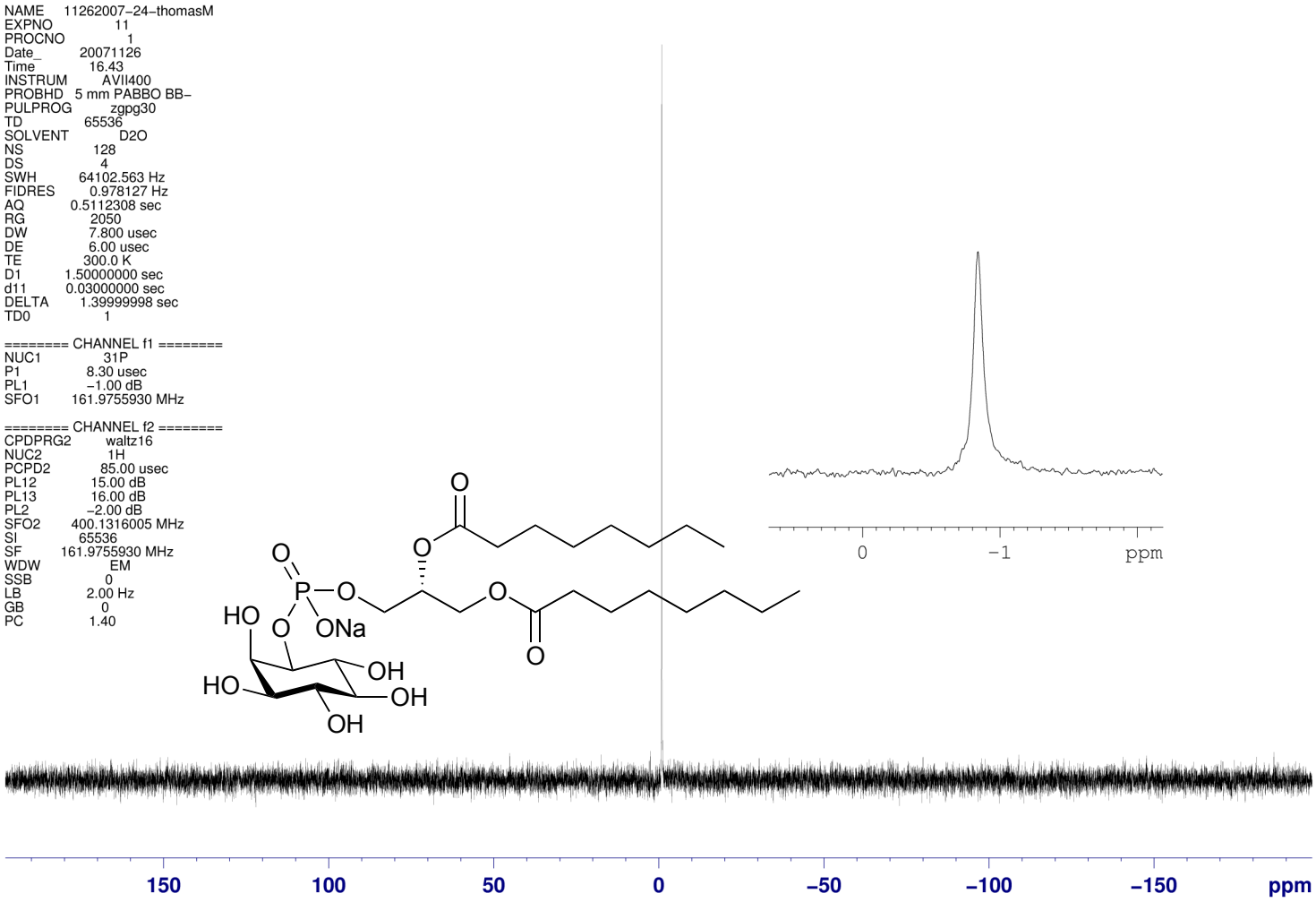
(+)-1D-Phosphatidylinositol 200



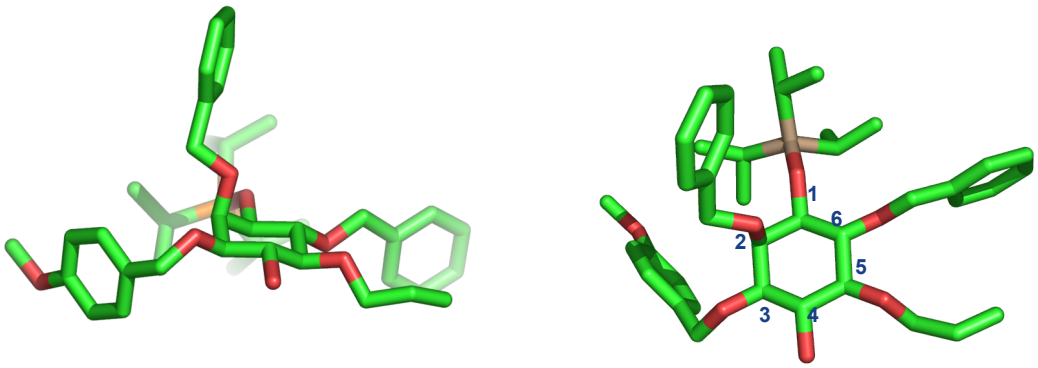
(+)-1D-Phosphatidylinositol 200



(+)-1D-Phosphatidylinositol 200



Appendix 2 – Crystallographic Data



X-Ray crystal structure of compound X

Table 1. Crystal data and structure refinement for compound **X**.

Identification code - tesc1			
Empirical formula	C40 H56 O7 Si	Theta range for data collection	1.78 to 25.32∞.
Formula weight	676.94	Index ranges	-13<=h<=12, -15<=k<=12, -34<=l<=32
Temperature	93(2) K	Reflections collected	24698
Wavelength	0.71073 ≈	Independent reflections	7017 [R(int) = 0.0674]
Crystal system	Monoclinic	Completeness to theta = 25.00∞	99.9 %
Space group	P2(1)/n	Absorption correction	Multiscan
Unit cell dimensions	a = 10.8233(15) ≈ a= 90∞. b = 12.5013(17) ≈ b= 90.238(4)∞. c = 28.564(4) ≈ g = 90∞.	Max. and min. transmission	1.0000 and 0.9733
Volume	3864.8(9) ≈^{3}	Refinement method	Full-matrix least-squares on F^2
Z	4	Data / restraints / parameters	7017 / 1 / 439
Density (calculated)	1.163 Mg/m^3	Goodness-of-fit on F^2	0.964
Absorption coefficient	0.107 mm^{-1}	Final R indices [I>2sigma(I)]	R1 = 0.0656, wR2 = 0.1696
F(000)	1464	R indices (all data)	R1 = 0.1015, wR2 = 0.1991
Crystal size	0.1500 x 0.0300 x 0.0300 mm^3	Largest diff. peak and hole	0.761 and -0.372 e.≈$^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (≈^{2}x 10^3) for tesc1. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Si(1)	3399(1)	1243(1)	3148(1)	26(1)
O(1)	4325(2)	2005(1)	3470(1)	28(1)
C(1)	4273(3)	2190(2)	3962(1)	25(1)
O(2)	4721(2)	4032(1)	3820(1)	28(1)
C(2)	3847(3)	3344(2)	4048(1)	25(1)
O(3)	3451(2)	4678(1)	4638(1)	31(1)
C(3)	3810(3)	3586(2)	4568(1)	28(1)
O(4)	5010(2)	3601(2)	5286(1)	33(1)
C(4)	5072(3)	3412(2)	4793(1)	27(1)
O(5)	6727(2)	2142(2)	4892(1)	33(1)
C(5)	5512(3)	2275(2)	4703(1)	28(1)
O(6)	5875(2)	905(1)	4127(1)	28(1)
C(6)	5542(2)	2005(2)	4185(1)	25(1)
C(7)	3568(3)	-195(2)	3347(1)	36(1)
C(8)	4783(3)	-696(2)	3184(1)	48(1)
C(9)	2462(3)	-915(2)	3218(1)	46(1)
C(10)	3960(3)	1555(2)	2540(1)	31(1)
C(11)	5356(3)	1495(3)	2466(1)	43(1)
C(12)	3274(3)	922(2)	2155(1)	39(1)
C(13)	1720(3)	1639(2)	3187(1)	29(1)
C(14)	1424(3)	2733(2)	2973(1)	39(1)
C(15)	1172(3)	1561(2)	3681(1)	36(1)
C(16)	4211(3)	4981(2)	3612(1)	36(1)
C(17)	4980(3)	5274(2)	3194(1)	30(1)
C(18)	5111(3)	4541(2)	2829(1)	37(1)
C(19)	5816(3)	4788(3)	2445(1)	48(1)
C(20)	6385(3)	5767(3)	2412(1)	53(1)
C(21)	6266(3)	6504(3)	2765(1)	55(1)
C(22)	5568(3)	6253(2)	3161(1)	42(1)
C(23)	2299(3)	4841(2)	4879(1)	32(1)
C(24)	1204(3)	4466(2)	4597(1)	29(1)
C(25)	891(3)	4974(2)	4180(1)	33(1)
C(26)	-99(3)	4630(2)	3907(1)	34(1)
O(27)	-1777(2)	3345(2)	3811(1)	42(1)
C(27)	-804(3)	3767(2)	4057(1)	31(1)
C(28)	-511(3)	3260(2)	4473(1)	33(1)
C(29)	476(3)	3607(2)	4741(1)	31(1)
C(30)	-2146(3)	3859(3)	3387(1)	48(1)
C(31)	6808(3)	1354(3)	5255(1)	54(1)
C(32)	8104(4)	1201(4)	5385(1)	72(1)
C(33)	8672(5)	263(5)	5317(2)	100(2)
C(34)	7117(3)	775(2)	3972(1)	33(1)
C(35)	7496(3)	-388(2)	3984(1)	28(1)
C(36)	6762(3)	-1187(2)	4172(1)	31(1)
C(37)	7163(3)	-2243(2)	4176(1)	37(1)
C(38)	8295(3)	-2513(2)	3997(1)	37(1)
C(39)	9043(3)	-1726(2)	3810(1)	40(1)
C(40)	8643(3)	-672(2)	3808(1)	32(1)

Table 3. Bond lengths [≈] and angles [∞] for tesc1.

Si(1)-O(1)	1.6585(19)	O(6)-C(6)-C(1)	108.9(2)
Si(1)-C(10)	1.881(3)	C(5)-C(6)-C(1)	110.4(2)
Si(1)-C(13)	1.887(3)	O(6)-C(6)-H(6A)	109.40
Si(1)-C(7)	1.894(3)	C(5)-C(6)-H(6A)	109.40
O(1)-C(1)	1.427(3)	C(1)-C(6)-H(6A)	109.40
C(1)-C(6)	1.528(4)	C(8)-C(7)-C(9)	110.9(2)
C(1)-C(2)	1.534(3)	C(8)-C(7)-Si(1)	112.3(2)
C(1)-H(1A)	1.0000	C(9)-C(7)-Si(1)	114.1(2)
O(2)-C(2)	1.435(3)	C(8)-C(7)-H(7A)	106.30
O(2)-C(16)	1.436(3)	C(9)-C(7)-H(7A)	106.30
C(2)-C(3)	1.516(4)	Si(1)-C(7)-H(7A)	106.30
C(2)-H(2A)	1.0000	C(7)-C(8)-H(8A)	109.50
O(3)-C(3)	1.433(3)	C(7)-C(8)-H(8B)	109.50
O(3)-C(23)	1.441(3)	H(8A)-C(8)-H(8B)	109.50
C(3)-C(4)	1.523(4)	C(7)-C(8)-H(8C)	109.50
C(3)-H(3A)	1.0000	H(8A)-C(8)-H(8C)	109.50
O(4)-C(4)	1.428(3)	H(8B)-C(8)-H(8C)	109.50
O(4)-H(4O)	0.939(19)	C(7)-C(9)-H(9A)	109.50
C(4)-C(5)	1.522(4)	C(7)-C(9)-H(9B)	109.50
C(4)-H(4A)	1.0000	H(9A)-C(9)-H(9B)	109.50
O(5)-C(5)	1.429(3)	C(7)-C(9)-H(9C)	109.50
O(5)-C(31)	1.434(4)	H(9A)-C(9)-H(9C)	109.50
C(5)-C(6)	1.520(4)	H(9B)-C(9)-H(9C)	109.50
C(5)-H(5A)	1.0000	C(11)-C(10)-C(12)	110.4(2)
O(6)-C(34)	1.426(3)	C(11)-C(10)-Si(1)	116.1(2)
O(6)-C(6)	1.432(3)	C(12)-C(10)-Si(1)	113.3(2)
C(6)-H(6A)	1.0000	C(11)-C(10)-H(10A)	105.30
C(7)-C(8)	1.531(4)	C(12)-C(10)-H(10A)	105.30
C(7)-C(9)	1.541(4)	Si(1)-C(10)-H(10A)	105.30
C(7)-H(7A)	1.0000	C(10)-C(11)-H(11A)	109.50
C(8)-H(8A)	0.9800	C(10)-C(11)-H(11B)	109.50
C(8)-H(8B)	0.9800	H(11A)-C(11)-H(11B)	109.50
C(8)-H(8C)	0.9800	C(10)-C(11)-H(11C)	109.50
C(9)-H(9A)	0.9800	H(11A)-C(11)-H(11C)	109.50
C(9)-H(9B)	0.9800	H(11B)-C(11)-H(11C)	109.50
C(9)-H(9C)	0.9800	C(10)-C(12)-H(12A)	109.50
C(10)-C(11)	1.528(4)	C(10)-C(12)-H(12B)	109.50
C(10)-C(12)	1.543(4)	H(12A)-C(12)-H(12B)	109.50
C(10)-H(10A)	1.0000	C(10)-C(12)-H(12C)	109.50
C(11)-H(11A)	0.9800	H(12A)-C(12)-H(12C)	109.50
C(11)-H(11B)	0.9800	H(12B)-C(12)-H(12C)	109.50
C(11)-H(11C)	0.9800	C(14)-C(13)-C(15)	110.0(2)
C(12)-H(12A)	0.9800	C(14)-C(13)-Si(1)	114.2(2)
C(12)-H(12B)	0.9800	C(15)-C(13)-Si(1)	114.4(2)
C(12)-H(12C)	0.9800	C(14)-C(13)-H(13A)	105.80
C(13)-C(14)	1.532(4)	C(15)-C(13)-H(13A)	105.80
C(13)-C(15)	1.538(4)	Si(1)-C(13)-H(13A)	105.80
C(13)-H(13A)	1.0000	C(13)-C(14)-H(14A)	109.50
C(14)-H(14A)	0.9800	C(13)-C(14)-H(14B)	109.50
C(14)-H(14B)	0.9800	H(14A)-C(14)-H(14B)	109.50

C(14)-H(14C)	0.9800
C(15)-H(15A)	0.9800
C(15)-H(15B)	0.9800
C(15)-H(15C)	0.9800
C(16)-C(17)	1.505(4)
C(16)-H(16A)	0.9900
C(16)-H(16B)	0.9900
C(17)-C(22)	1.383(4)
C(17)-C(18)	1.394(4)
C(18)-C(19)	1.375(4)
C(18)-H(18A)	0.9500
C(19)-C(20)	1.374(5)
C(19)-H(19A)	0.9500
C(20)-C(21)	1.372(5)
C(20)-H(20A)	0.9500
C(21)-C(22)	1.398(5)
C(21)-H(21A)	0.9500
C(22)-H(22A)	0.9500
C(23)-C(24)	1.504(4)
C(23)-H(23A)	0.9900
C(23)-H(23B)	0.9900
C(24)-C(25)	1.390(4)
C(24)-C(29)	1.395(4)
C(25)-C(26)	1.392(4)
C(25)-H(25A)	0.9500
C(26)-C(27)	1.389(4)
C(26)-H(26A)	0.9500
O(27)-C(27)	1.370(3)
O(27)-C(30)	1.427(4)
C(27)-C(28)	1.383(4)
C(28)-C(29)	1.380(4)
C(28)-H(28A)	0.9500
C(29)-H(29A)	0.9500
C(30)-H(30A)	0.9800
C(30)-H(30B)	0.9800
C(30)-H(30C)	0.9800
C(31)-C(32)	1.462(5)
C(31)-H(31A)	0.9900
C(31)-H(31B)	0.9900
C(32)-C(33)	1.339(6)
C(32)-H(32A)	0.9500
C(33)-H(33A)	0.9500
C(33)-H(33B)	0.9500
C(34)-C(35)	1.510(4)
C(34)-H(34A)	0.9900
C(34)-H(34B)	0.9900
C(35)-C(36)	1.386(4)
C(35)-C(40)	1.388(4)
C(36)-C(37)	1.389(4)
C(36)-H(36A)	0.9500
C(37)-C(38)	1.373(4)
C(37)-H(37A)	0.9500
C(38)-C(39)	1.382(4)
C(38)-H(38A)	0.9500
C(39)-C(40)	1.388(4)
C(39)-H(39A)	0.9500

C(13)-C(14)-H(14C)	109.50
H(14A)-C(14)-H(14C)	109.50
H(14B)-C(14)-H(14C)	109.50
C(13)-C(15)-H(15A)	109.50
C(13)-C(15)-H(15B)	109.50
H(15A)-C(15)-H(15B)	109.50
C(13)-C(15)-H(15C)	109.50
H(15A)-C(15)-H(15C)	109.50
H(15B)-C(15)-H(15C)	109.50
O(2)-C(16)-C(17)	108.5(2)
O(2)-C(16)-H(16A)	110.00
C(17)-C(16)-H(16A)	110.00
O(2)-C(16)-H(16B)	110.00
C(17)-C(16)-H(16B)	110.00
H(16A)-C(16)-H(16B)	108.40
C(22)-C(17)-C(18)	118.9(3)
C(22)-C(17)-C(16)	121.7(3)
C(18)-C(17)-C(16)	119.4(3)
C(19)-C(18)-C(17)	120.5(3)
C(19)-C(18)-H(18A)	119.80
C(17)-C(18)-H(18A)	119.80
C(20)-C(19)-C(18)	120.3(3)
C(20)-C(19)-H(19A)	119.90
C(18)-C(19)-H(19A)	119.90
C(21)-C(20)-C(19)	120.4(3)
C(21)-C(20)-H(20A)	119.80
C(19)-C(20)-H(20A)	119.80
C(20)-C(21)-C(22)	119.8(3)
C(20)-C(21)-H(21A)	120.10
C(22)-C(21)-H(21A)	120.10
C(17)-C(22)-C(21)	120.2(3)
C(17)-C(22)-H(22A)	119.90
C(21)-C(22)-H(22A)	119.90
O(3)-C(23)-C(24)	112.4(2)
O(3)-C(23)-H(23A)	109.10
C(24)-C(23)-H(23A)	109.10
O(3)-C(23)-H(23B)	109.10
C(24)-C(23)-H(23B)	109.10
H(23A)-C(23)-H(23B)	107.90
C(25)-C(24)-C(29)	117.9(3)
C(25)-C(24)-C(23)	120.3(3)
C(29)-C(24)-C(23)	121.8(3)
C(24)-C(25)-C(26)	121.6(3)
C(24)-C(25)-H(25A)	119.20
C(26)-C(25)-H(25A)	119.20
C(27)-C(26)-C(25)	119.3(3)
C(27)-C(26)-H(26A)	120.30
C(25)-C(26)-H(26A)	120.30
C(27)-O(27)-C(30)	118.3(2)
O(27)-C(27)-C(28)	115.9(2)
O(27)-C(27)-C(26)	124.3(3)
C(28)-C(27)-C(26)	119.7(3)
C(29)-C(28)-C(27)	120.4(3)
C(29)-C(28)-H(28A)	119.80
C(27)-C(28)-H(28A)	119.80
C(28)-C(29)-C(24)	121.1(3)

C(40)-H(40A)	0.9500	C(28)-C(29)-H(29A)	119.50
		C(24)-C(29)-H(29A)	119.50
O(1)-Si(1)-C(10)	101.31(11)	O(27)-C(30)-H(30A)	109.50
O(1)-Si(1)-C(13)	113.34(11)	O(27)-C(30)-H(30B)	109.50
C(10)-Si(1)-C(13)	108.32(13)	H(30A)-C(30)-H(30B)	109.50
O(1)-Si(1)-C(7)	108.74(12)	O(27)-C(30)-H(30C)	109.50
C(10)-Si(1)-C(7)	116.29(13)	H(30A)-C(30)-H(30C)	109.50
C(13)-Si(1)-C(7)	108.83(13)	H(30B)-C(30)-H(30C)	109.50
C(1)-O(1)-Si(1)	127.88(17)	O(5)-C(31)-C(32)	109.3(3)
O(1)-C(1)-C(6)	110.3(2)	O(5)-C(31)-H(31A)	109.80
O(1)-C(1)-C(2)	108.8(2)	C(32)-C(31)-H(31A)	109.80
C(6)-C(1)-C(2)	110.3(2)	O(5)-C(31)-H(31B)	109.80
O(1)-C(1)-H(1A)	109.20	C(32)-C(31)-H(31B)	109.80
C(6)-C(1)-H(1A)	109.20	H(31A)-C(31)-H(31B)	108.30
C(2)-C(1)-H(1A)	109.20	C(33)-C(32)-C(31)	121.2(5)
C(2)-O(2)-C(16)	115.4(2)	C(33)-C(32)-H(32A)	119.40
O(2)-C(2)-C(3)	110.1(2)	C(31)-C(32)-H(32A)	119.40
O(2)-C(2)-C(1)	107.0(2)	C(32)-C(33)-H(33A)	120.00
C(3)-C(2)-C(1)	110.6(2)	C(32)-C(33)-H(33B)	120.00
O(2)-C(2)-H(2A)	109.70	H(33A)-C(33)-H(33B)	120.00
C(3)-C(2)-H(2A)	109.70	O(6)-C(34)-C(35)	111.0(2)
C(1)-C(2)-H(2A)	109.70	O(6)-C(34)-H(34A)	109.40
C(3)-O(3)-C(23)	115.9(2)	C(35)-C(34)-H(34A)	109.40
O(3)-C(3)-C(2)	109.5(2)	O(6)-C(34)-H(34B)	109.40
O(3)-C(3)-C(4)	108.7(2)	C(35)-C(34)-H(34B)	109.40
C(2)-C(3)-C(4)	111.0(2)	H(34A)-C(34)-H(34B)	108.00
O(3)-C(3)-H(3A)	109.20	C(36)-C(35)-C(40)	118.1(2)
C(2)-C(3)-H(3A)	109.20	C(36)-C(35)-C(34)	123.2(3)
C(4)-C(3)-H(3A)	109.20	C(40)-C(35)-C(34)	118.7(2)
C(4)-O(4)-H(4O)	105(2)	C(35)-C(36)-C(37)	120.6(3)
O(4)-C(4)-C(5)	109.7(2)	C(35)-C(36)-H(36A)	119.70
O(4)-C(4)-C(3)	110.3(2)	C(37)-C(36)-H(36A)	119.70
C(5)-C(4)-C(3)	110.0(2)	C(38)-C(37)-C(36)	120.7(3)
O(4)-C(4)-H(4A)	108.90	C(38)-C(37)-H(37A)	119.70
C(5)-C(4)-H(4A)	108.90	C(36)-C(37)-H(37A)	119.70
C(3)-C(4)-H(4A)	108.90	C(37)-C(38)-C(39)	119.6(3)
C(5)-O(5)-C(31)	114.0(2)	C(37)-C(38)-H(38A)	120.20
O(5)-C(5)-C(6)	108.5(2)	C(39)-C(38)-H(38A)	120.20
O(5)-C(5)-C(4)	109.4(2)	C(38)-C(39)-C(40)	119.7(3)
C(6)-C(5)-C(4)	112.4(2)	C(38)-C(39)-H(39A)	120.20
O(5)-C(5)-H(5A)	108.80	C(40)-C(39)-H(39A)	120.20
C(6)-C(5)-H(5A)	108.80	C(39)-C(40)-C(35)	121.4(3)
C(4)-C(5)-H(5A)	108.80	C(39)-C(40)-H(40A)	119.30
C(34)-O(6)-C(6)	112.56(19)	C(35)-C(40)-H(40A)	119.30
O(6)-C(6)-C(5)	109.4(2)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($\approx^2$x 10^3) for tesc1. The anisotropic displacement factor exponent takes the form: $-2p^2[\, h^2\, a^{*2}U^{11} + \ldots + 2\, h\, k\, a^*\, b^*\, U^{12}\,]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Si(1)	28(1)	24(1)	27(1)	-4(1)	-4(1)	2(1)
O(1)	31(1)	28(1)	23(1)	-7(1)	-3(1)	1(1)
C(1)	27(2)	26(1)	23(1)	-6(1)	-1(1)	-1(1)
O(2)	25(1)	26(1)	32(1)	1(1)	-2(1)	1(1)
C(2)	21(2)	26(1)	29(2)	-5(1)	-4(1)	-2(1)
O(3)	27(1)	27(1)	40(1)	-11(1)	6(1)	-2(1)
C(3)	28(2)	27(1)	28(2)	-7(1)	4(1)	-4(1)
O(4)	40(1)	37(1)	22(1)	-6(1)	4(1)	-15(1)
C(4)	27(2)	34(1)	20(1)	-4(1)	0(1)	-7(1)
O(5)	27(1)	46(1)	28(1)	5(1)	-7(1)	-3(1)
C(5)	22(2)	33(1)	30(2)	-2(1)	-2(1)	-5(1)
O(6)	24(1)	24(1)	36(1)	-2(1)	0(1)	2(1)
C(6)	26(2)	24(1)	26(1)	-1(1)	-2(1)	1(1)
C(7)	47(2)	24(1)	35(2)	-3(1)	-8(2)	1(1)
C(8)	48(2)	34(2)	63(2)	-9(2)	-16(2)	13(1)
C(9)	57(2)	30(2)	50(2)	-4(2)	0(2)	-4(2)
C(10)	32(2)	31(1)	30(2)	-4(1)	-4(1)	2(1)
C(11)	39(2)	53(2)	38(2)	-3(2)	-1(2)	-2(2)
C(12)	43(2)	45(2)	29(2)	-8(1)	-5(1)	2(1)
C(13)	27(2)	29(1)	33(2)	-5(1)	-3(1)	-1(1)
C(14)	38(2)	33(2)	45(2)	4(1)	-4(2)	10(1)
C(15)	29(2)	39(2)	40(2)	-2(1)	1(1)	-2(1)
C(16)	44(2)	31(1)	33(2)	1(1)	0(1)	11(1)
C(17)	27(2)	31(1)	32(2)	1(1)	-4(1)	6(1)
C(18)	34(2)	45(2)	31(2)	-2(1)	-5(1)	1(1)
C(19)	43(2)	66(2)	35(2)	-3(2)	0(2)	6(2)
C(20)	37(2)	71(2)	53(2)	22(2)	7(2)	3(2)
C(21)	41(2)	46(2)	77(3)	20(2)	-1(2)	-6(2)
C(22)	40(2)	33(2)	53(2)	-2(2)	-6(2)	-1(1)
C(23)	26(2)	37(2)	34(2)	-10(1)	5(1)	-2(1)
C(24)	26(2)	29(1)	34(2)	-9(1)	4(1)	2(1)
C(25)	29(2)	29(1)	42(2)	-1(1)	7(1)	0(1)
C(26)	31(2)	31(1)	40(2)	7(1)	4(1)	6(1)
O(27)	31(1)	47(1)	46(1)	5(1)	-10(1)	-6(1)
C(27)	26(2)	29(1)	38(2)	-1(1)	-1(1)	4(1)
C(28)	30(2)	31(1)	39(2)	-3(1)	7(1)	-3(1)
C(29)	29(2)	34(1)	30(2)	-4(1)	5(1)	0(1)
C(30)	34(2)	58(2)	53(2)	10(2)	-16(2)	-2(2)
C(31)	37(2)	87(3)	39(2)	27(2)	0(2)	8(2)
C(32)	46(3)	114(4)	55(2)	27(2)	-2(2)	-4(2)
C(33)	106(4)	139(4)	55(3)	36(3)	8(3)	72(4)
C(34)	26(2)	34(2)	38(2)	2(1)	4(1)	4(1)
C(35)	24(2)	33(1)	27(2)	3(1)	-2(1)	1(1)
C(36)	27(2)	32(1)	33(2)	4(1)	3(1)	2(1)
C(37)	44(2)	31(2)	36(2)	7(1)	2(1)	-7(1)
C(38)	42(2)	25(1)	44(2)	2(1)	5(2)	8(1)
C(39)	36(2)	36(2)	47(2)	-3(1)	6(2)	8(1)
C(40)	30(2)	31(1)	35(2)	0(1)	4(1)	-1(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (≈^{2}x 10 ^{3}) for tesc1.

	x	y	z	U(eq)
H(1A)	3664	1686	4105	30
H(2A)	3008	3453	3908	30
H(3A)	3193	3106	4721	33
H(4O)	5520(30)	4200(20)	5340(13)	72(12)
H(4A)	5675	3924	4652	32
H(5A)	4941	1766	4864	34
H(6A)	6166	2467	4026	30
H(7A)	3607	-176	3697	43
H(8A)	4834	-1435	3297	72
H(8B)	5479	-283	3310	72
H(8C)	4813	-690	2841	72
H(9A)	2610	-1641	3335	69
H(9B)	2362	-933	2877	69
H(9C)	1709	-628	3361	69
H(10A)	3736	2321	2486	37
H(11A)	5548	1680	2141	65
H(11B)	5645	768	2533	65
H(11C)	5770	2000	2677	65
H(12A)	3601	1122	1848	59
H(12B)	2390	1089	2167	59
H(12C)	3395	154	2206	59
H(13A)	1257	1105	2993	35
H(14A)	539	2879	3004	58
H(14B)	1648	2733	2641	58
H(14C)	1896	3288	3137	58
H(15A)	301	1774	3672	54
H(15B)	1628	2038	3893	54
H(15C)	1239	823	3794	54
H(16A)	4218	5573	3842	43
H(16B)	3345	4850	3515	43
H(18A)	4710	3866	2847	44
H(19A)	5911	4279	2201	57
H(20A)	6862	5935	2144	64
H(21A)	6657	7182	2741	66
H(22A)	5498	6757	3409	50
H(23A)	2202	5611	4949	38
H(23B)	2322	4449	5180	38
H(25A)	1365	5571	4080	40
H(26A)	-291	4981	3621	40
H(28A)	-992	2670	4576	40
H(29A)	662	3254	5027	37
H(30A)	-2348	4609	3451	72
H(30B)	-2874	3497	3257	72
H(30C)	-1469	3825	3160	72
H(31A)	6454	669	5144	65
H(31B)	6332	1592	5531	65
H(32A)	8551	1778	5521	86
H(33A)	8233	-318	5182	120
H(33B)	9515	179	5404	120
H(34A)	7677	1197	4175	40
H(34B)	7194	1050	3648	40
H(36A)	5977	-1012	4299	37

H(37A)	6649	-2783	4305	44
H(38A)	8562	-3237	4000	44
H(39A)	9826	-1907	3684	48
H(40A)	9165	-133	3683	38

Table 6. Torsion angles [∞] for tesc1.

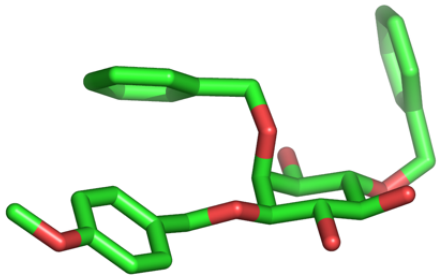
C(10)-Si(1)-O(1)-C(1)	173.7(2)	C(13)-Si(1)-C(10)-C(12)	-62.1(2)
C(13)-Si(1)-O(1)-C(1)	57.9(2)	C(7)-Si(1)-C(10)-C(12)	60.8(2)
C(7)-Si(1)-O(1)-C(1)	-63.3(2)	O(1)-Si(1)-C(13)-C(14)	66.8(2)
Si(1)-O(1)-C(1)-C(6)	129.44(19)	C(10)-Si(1)-C(13)-C(14)	-44.8(2)
Si(1)-O(1)-C(1)-C(2)	-109.5(2)	C(7)-Si(1)-C(13)-C(14)	-172.1(2)
C(16)-O(2)-C(2)-C(3)	-95.5(3)	O(1)-Si(1)-C(13)-C(15)	-61.2(2)
C(16)-O(2)-C(2)-C(1)	144.2(2)	C(10)-Si(1)-C(13)-C(15)	-172.79(18)
O(1)-C(1)-C(2)-O(2)	-58.6(3)	C(7)-Si(1)-C(13)-C(15)	59.9(2)
C(6)-C(1)-C(2)-O(2)	62.4(3)	C(2)-O(2)-C(16)-C(17)	-149.3(2)
O(1)-C(1)-C(2)-C(3)	-178.5(2)	O(2)-C(16)-C(17)-C(22)	-121.0(3)
C(6)-C(1)-C(2)-C(3)	-57.5(3)	O(2)-C(16)-C(17)-C(18)	58.6(3)
C(23)-O(3)-C(3)-C(2)	116.4(2)	C(22)-C(17)-C(18)-C(19)	0.2(5)
C(23)-O(3)-C(3)-C(4)	-122.1(2)	C(16)-C(17)-C(18)-C(19)	-179.5(3)
O(2)-C(2)-C(3)-O(3)	60.0(3)	C(17)-C(18)-C(19)-C(20)	-1.0(5)
C(1)-C(2)-C(3)-O(3)	178.1(2)	C(18)-C(19)-C(20)-C(21)	0.8(5)
O(2)-C(2)-C(3)-C(4)	-60.0(3)	C(19)-C(20)-C(21)-C(22)	0.3(5)
C(1)-C(2)-C(3)-C(4)	58.0(3)	C(18)-C(17)-C(22)-C(21)	0.9(5)
O(3)-C(3)-C(4)-O(4)	61.8(3)	C(16)-C(17)-C(22)-C(21)	-179.4(3)
C(2)-C(3)-C(4)-O(4)	-177.62(19)	C(20)-C(21)-C(22)-C(17)	-1.2(5)
O(3)-C(3)-C(4)-C(5)	-177.0(2)	C(3)-O(3)-C(23)-C(24)	-67.1(3)
C(2)-C(3)-C(4)-C(5)	-56.5(3)	O(3)-C(23)-C(24)-C(25)	-65.6(3)
C(31)-O(5)-C(5)-C(6)	-119.2(3)	O(3)-C(23)-C(24)-C(29)	114.2(3)
C(31)-O(5)-C(5)-C(4)	117.9(3)	C(29)-C(24)-C(25)-C(26)	-1.3(4)
O(4)-C(4)-C(5)-O(5)	-62.0(3)	C(23)-C(24)-C(25)-C(26)	178.5(2)
C(3)-C(4)-C(5)-O(5)	176.5(2)	C(24)-C(25)-C(26)-C(27)	1.0(4)
O(4)-C(4)-C(5)-C(6)	177.4(2)	C(30)-O(27)-C(27)-C(28)	177.9(3)
C(3)-C(4)-C(5)-C(6)	55.8(3)	C(30)-O(27)-C(27)-C(26)	-4.0(4)
C(34)-O(6)-C(6)-C(5)	-104.9(2)	C(25)-C(26)-C(27)-O(27)	-178.3(3)
C(34)-O(6)-C(6)-C(1)	134.3(2)	C(25)-C(26)-C(27)-C(28)	-0.3(4)
O(5)-C(5)-C(6)-O(6)	63.0(3)	O(27)-C(27)-C(28)-C(29)	178.3(2)
C(4)-C(5)-C(6)-O(6)	-175.8(2)	C(26)-C(27)-C(28)-C(29)	0.1(4)
O(5)-C(5)-C(6)-C(1)	-177.1(2)	C(27)-C(28)-C(29)-C(24)	-0.5(4)
C(4)-C(5)-C(6)-C(1)	-56.0(3)	C(25)-C(24)-C(29)-C(28)	1.1(4)
O(1)-C(1)-C(6)-O(6)	-63.7(3)	C(23)-C(24)-C(29)-C(28)	-178.7(3)
C(2)-C(1)-C(6)-O(6)	176.1(2)	C(5)-O(5)-C(31)-C(32)	173.9(3)
O(1)-C(1)-C(6)-C(5)	176.11(19)	O(5)-C(31)-C(32)-C(33)	-115.9(4)
C(2)-C(1)-C(6)-C(5)	55.9(3)	C(6)-O(6)-C(34)-C(35)	171.5(2)
O(1)-Si(1)-C(7)-C(8)	-74.4(2)	O(6)-C(34)-C(35)-C(36)	-6.5(4)
C(10)-Si(1)-C(7)-C(8)	39.1(3)	O(6)-C(34)-C(35)-C(40)	174.7(2)
C(13)-Si(1)-C(7)-C(8)	161.7(2)	C(40)-C(35)-C(36)-C(37)	-0.8(4)
O(1)-Si(1)-C(7)-C(9)	158.4(2)	C(34)-C(35)-C(36)-C(37)	-179.6(3)
C(10)-Si(1)-C(7)-C(9)	-88.1(3)	C(35)-C(36)-C(37)-C(38)	0.2(4)
C(13)-Si(1)-C(7)-C(9)	34.5(3)	C(36)-C(37)-C(38)-C(39)	0.1(5)
O(1)-Si(1)-C(10)-C(11)	49.1(2)	C(37)-C(38)-C(39)-C(40)	0.2(5)
C(13)-Si(1)-C(10)-C(11)	168.5(2)	C(38)-C(39)-C(40)-C(35)	-0.8(5)
C(7)-Si(1)-C(10)-C(11)	-68.6(2)	C(36)-C(35)-C(40)-C(39)	1.2(4)
O(1)-Si(1)-C(10)-C(12)	178.5(2)	C(34)-C(35)-C(40)-C(39)	180.0(3)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for tesc1 [≈ and ∞].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(4)-H(4O)...O(3)#1	0.939(19)	1.797(19)	2.730(3)	172(3)

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,-y+1,-z+1



X-Ray crystal structure of compound X

Table 1. Crystal data and structure refinement for **X**.

Identification code - tesc2

Empirical formula	C28 H32 O7	Theta range for data collection	2.97 to 25.40∞.
Formula weight	480.54	Index ranges	-7<=h<=7, -27<=k<=26, -20<=l<=20
Temperature	93(2) K	Reflections collected	24997
Wavelength	0.71073 ≈	Independent reflections	8888 [R(int) = 0.0851]
Crystal system	Monoclinic	Completeness to theta = 25.00∞	99.8 %
Space group	P2(1)	Absorption correction	Multiscan
Unit cell dimensions	a = 6.500(2) ≈ a= 90∞. b = 22.510(8) ≈ b= 93.104(5)∞. c = 16.801(6) ≈ g = 90∞.	Max. and min. transmission	1.0000 and 0.9749
Volume	2454.8(15) ≈^{3}	Refinement method	Full-matrix least-squares on F^2
Z	4	Data / restraints / parameters	8888 / 7 / 659
Density (calculated)	1.300 Mg/m^3	Goodness-of-fit on F^2	1.077
Absorption coefficient	0.093 mm^{-1}	Final R indices [I>2sigma(I)]	R1 = 0.0736, wR2 = 0.1636
F(000)	1024	R indices (all data)	R1 = 0.0903, wR2 = 0.1757
Crystal size	0.3000 x 0.0300 x 0.0100 mm^3	Absolute structure parameter	1.2(10)

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (≈^{2}x 10^3) for tesc2. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	4816(4)	4369(1)	4369(2)	40(1)
C(1)	3265(6)	4168(2)	3789(2)	36(1)
O(2)	279(4)	4340(1)	4605(2)	36(1)
C(2)	1260(6)	4504(2)	3890(2)	33(1)
O(3)	-2218(4)	4634(1)	3261(2)	36(1)
C(3)	-317(5)	4322(2)	3218(2)	33(1)
O(4)	-2215(4)	3476(1)	2667(2)	36(1)
C(4)	-771(6)	3667(2)	3301(2)	34(1)
O(5)	617(4)	2692(1)	3502(2)	37(1)
C(5)	1159(6)	3288(2)	3289(2)	34(1)
O(6)	4775(4)	3197(1)	3714(2)	37(1)
C(6)	2888(5)	3506(2)	3871(2)	32(1)
C(7)	1142(6)	4582(2)	5343(2)	39(1)
C(8)	837(6)	5244(2)	5395(2)	40(1)
C(9)	-1103(7)	5486(2)	5477(3)	52(1)
C(10)	-1374(8)	6090(2)	5545(3)	59(1)
C(11)	279(8)	6468(2)	5528(3)	54(1)
C(12)	2225(8)	6238(2)	5433(3)	62(1)
C(13)	2495(7)	5632(2)	5366(3)	51(1)
C(14)	-2043(6)	5259(2)	3090(3)	44(1)
C(15)	-4131(6)	5539(2)	2985(2)	38(1)
C(16)	-5542(6)	5368(2)	2372(3)	41(1)
C(17)	-7389(6)	5664(2)	2245(2)	38(1)
O(18)	-9723(4)	6411(1)	2557(2)	45(1)
C(18)	-7858(6)	6135(2)	2737(3)	38(1)
C(19)	-6504(6)	6304(2)	3354(3)	43(1)
C(20)	-4660(6)	6003(2)	3478(3)	43(1)
C(21)	-10236(7)	6899(2)	3057(4)	64(1)
C(22)	5274(6)	2686(2)	4212(2)	40(1)
C(23)	5717(6)	2839(2)	5075(2)	39(1)
C(24)	4216(7)	2791(2)	5631(3)	45(1)
C(25)	4619(7)	2968(2)	6417(3)	48(1)
C(26)	6523(8)	3200(2)	6654(3)	53(1)
C(27)	8034(7)	3235(2)	6118(3)	52(1)
C(28)	7635(6)	3059(2)	5334(3)	45(1)
O(15)	-8475(4)	3744(1)	7889(2)	47(1)
C(30)	7436(7)	7110(2)	10197(3)	52(1)
O(31)	5697(4)	6058(1)	8903(2)	41(1)
C(31)	4041(6)	6251(2)	8361(3)	39(1)
O(32)	1511(4)	5789(1)	9193(2)	42(1)
C(32)	2254(6)	5823(2)	8406(2)	36(1)
O(33)	-1361(4)	5657(1)	7937(2)	40(1)
C(33)	416(6)	6025(2)	7869(2)	36(1)
O(34)	-1865(4)	6857(1)	7557(2)	37(1)
C(34)	-235(6)	6653(2)	8095(2)	36(1)
O(35)	876(4)	7648(1)	8380(2)	39(1)
C(35)	1566(6)	7087(2)	8089(2)	34(1)
O(36)	5140(4)	7263(1)	8514(2)	38(1)
C(36)	3418(5)	6871(2)	8603(2)	34(1)
C(37)	2592(6)	5386(2)	9734(3)	47(1)
C(38)	1043(6)	5027(2)	10164(2)	38(1)
C(39)	1603(7)	4478(2)	10493(3)	47(1)

C(40)	243(8)	4148(2)	10918(3)	57(1)
C(41)	-1722(7)	4364(2)	11025(3)	54(1)
C(42)	-2281(7)	4904(2)	10692(3)	52(1)
C(43)	-920(6)	5239(2)	10260(3)	43(1)
C(44)	-1006(6)	5039(2)	7793(3)	48(1)
C(45)	-3015(6)	4704(2)	7842(3)	42(1)
C(46)	-3347(6)	4348(2)	8500(3)	43(1)
C(47)	-5163(6)	4024(2)	8547(3)	45(1)
C(48)	-6649(6)	4050(2)	7923(2)	39(1)
C(49)	-6333(6)	4413(2)	7274(2)	44(1)
C(50)	-4520(6)	4736(2)	7242(3)	43(1)
C(51)	-8834(7)	3354(2)	8541(3)	56(1)
C(52)	5489(7)	7708(2)	9133(2)	43(1)
C(53)	5677(7)	7438(2)	9955(3)	44(1)
C(54)	4055(7)	7471(2)	10467(3)	52(1)
C(55)	4157(9)	7172(3)	11186(3)	68(2)
C(56)	5861(10)	6844(3)	11411(3)	70(2)
C(57)	7512(10)	6812(2)	10923(3)	66(2)

Table 3. Bond lengths [≈] and angles [∞] for tesc2.

O(1)-C(1)	1.437(4)	C(10)-C(9)-C(8)	121.1(4)
O(1)-H(1O)	0.961(19)	C(10)-C(9)-H(9A)	119.4000
C(1)-C(6)	1.517(5)	C(8)-C(9)-H(9A)	119.4000
C(1)-C(2)	1.524(5)	C(11)-C(10)-C(9)	120.5(4)
C(1)-H(1A)	1.0000	C(11)-C(10)-H(10A)	119.8000
O(2)-C(2)	1.438(4)	C(9)-C(10)-H(10A)	119.8000
O(2)-C(7)	1.439(5)	C(10)-C(11)-C(12)	119.4(4)
C(2)-C(3)	1.538(5)	C(10)-C(11)-H(11A)	120.3000
C(2)-H(2A)	1.0000	C(12)-C(11)-H(11A)	120.3000
O(3)-C(3)	1.427(4)	C(13)-C(12)-C(11)	120.0(5)
O(3)-C(14)	1.442(5)	C(13)-C(12)-H(12A)	120.0000
C(3)-C(4)	1.511(5)	C(11)-C(12)-H(12A)	120.0000
C(3)-H(3A)	1.0000	C(12)-C(13)-C(8)	121.0(4)
O(4)-C(4)	1.447(4)	C(12)-C(13)-H(13A)	119.5000
O(4)-H(4O)	0.97(2)	C(8)-C(13)-H(13A)	119.5000
C(4)-C(5)	1.519(5)	O(3)-C(14)-C(15)	110.6(3)
C(4)-H(4A)	1.0000	O(3)-C(14)-H(14A)	109.5000
O(5)-C(5)	1.436(5)	C(15)-C(14)-H(14A)	109.5000
O(5)-H(5O)	0.97(2)	O(3)-C(14)-H(14B)	109.5000
C(5)-C(6)	1.531(5)	C(15)-C(14)-H(14B)	109.5000
C(5)-H(5A)	1.0000	H(14A)-C(14)-H(14B)	108.1000
O(6)-C(6)	1.447(4)	C(20)-C(15)-C(16)	118.3(4)
O(6)-C(22)	1.449(5)	C(20)-C(15)-C(14)	119.9(4)
C(6)-H(6A)	1.0000	C(16)-C(15)-C(14)	121.7(4)
C(7)-C(8)	1.507(6)	C(17)-C(16)-C(15)	120.8(4)
C(7)-H(7A)	0.9900	C(17)-C(16)-H(16A)	119.6000
C(7)-H(7B)	0.9900	C(15)-C(16)-H(16A)	119.6000
C(8)-C(9)	1.387(6)	C(16)-C(17)-C(18)	119.7(4)
C(8)-C(13)	1.390(6)	C(16)-C(17)-H(17A)	120.2000
C(9)-C(10)	1.377(7)	C(18)-C(17)-H(17A)	120.2000
C(9)-H(9A)	0.9500	C(18)-O(18)-C(21)	116.6(3)
C(10)-C(11)	1.373(7)	C(19)-C(18)-O(18)	123.9(4)
C(10)-H(10A)	0.9500	C(19)-C(18)-C(17)	120.4(4)
C(11)-C(12)	1.384(7)	O(18)-C(18)-C(17)	115.6(3)
C(11)-H(11A)	0.9500	C(18)-C(19)-C(20)	119.5(4)
C(12)-C(13)	1.382(7)	C(18)-C(19)-H(19A)	120.2000
C(12)-H(12A)	0.9500	C(20)-C(19)-H(19A)	120.2000
C(13)-H(13A)	0.9500	C(19)-C(20)-C(15)	121.3(4)
C(14)-C(15)	1.498(5)	C(19)-C(20)-H(20A)	119.4000
C(14)-H(14A)	0.9900	C(15)-C(20)-H(20A)	119.4000
C(14)-H(14B)	0.9900	O(18)-C(21)-H(21A)	109.5000
C(15)-C(20)	1.388(6)	O(18)-C(21)-H(21B)	109.5000
C(15)-C(16)	1.396(6)	H(21A)-C(21)-H(21B)	109.5000
C(16)-C(17)	1.380(5)	O(18)-C(21)-H(21C)	109.5000
C(16)-H(16A)	0.9500	H(21A)-C(21)-H(21C)	109.5000
C(17)-C(18)	1.388(6)	H(21B)-C(21)-H(21C)	109.5000
C(17)-H(17A)	0.9500	O(6)-C(22)-C(23)	113.6(3)
O(18)-C(18)	1.381(4)	O(6)-C(22)-H(22A)	108.8000
O(18)-C(21)	1.433(6)	C(23)-C(22)-H(22A)	108.8000
C(18)-C(19)	1.377(6)	O(6)-C(22)-H(22B)	108.8000
C(19)-C(20)	1.383(6)	C(23)-C(22)-H(22B)	108.8000
C(19)-H(19A)	0.9500	H(22A)-C(22)-H(22B)	107.7000
C(20)-H(20A)	0.9500	C(28)-C(23)-C(24)	117.9(4)
C(21)-H(21A)	0.9800	C(28)-C(23)-C(22)	120.4(4)

C(21)-H(21B)	0.9800	C(24)-C(23)-C(22)	121.6(4)
C(21)-H(21C)	0.9800	C(25)-C(24)-C(23)	120.8(4)
C(22)-C(23)	1.503(6)	C(25)-C(24)-H(24A)	119.6000
C(22)-H(22A)	0.9900	C(23)-C(24)-H(24A)	119.6000
C(22)-H(22B)	0.9900	C(26)-C(25)-C(24)	120.1(4)
C(23)-C(28)	1.389(6)	C(26)-C(25)-H(25A)	119.9000
C(23)-C(24)	1.391(6)	C(24)-C(25)-H(25A)	119.9000
C(24)-C(25)	1.390(6)	C(27)-C(26)-C(25)	119.6(4)
C(24)-H(24A)	0.9500	C(27)-C(26)-H(26A)	120.2000
C(25)-C(26)	1.382(7)	C(25)-C(26)-H(26A)	120.2000
C(25)-H(25A)	0.9500	C(26)-C(27)-C(28)	120.3(4)
C(26)-C(27)	1.371(7)	C(26)-C(27)-H(27A)	119.8000
C(26)-H(26A)	0.9500	C(28)-C(27)-H(27A)	119.8000
C(27)-C(28)	1.386(6)	C(27)-C(28)-C(23)	121.1(4)
C(27)-H(27A)	0.9500	C(27)-C(28)-H(28A)	119.4000
C(28)-H(28A)	0.9500	C(23)-C(28)-H(28A)	119.4000
O(15)-C(48)	1.371(5)	C(48)-O(15)-C(51)	116.9(3)
O(15)-C(51)	1.431(5)	C(57)-C(30)-C(53)	119.9(5)
C(30)-C(57)	1.389(7)	C(57)-C(30)-H(30A)	120.1000
C(30)-C(53)	1.402(6)	C(53)-C(30)-H(30A)	120.1000
C(30)-H(30A)	0.9500	C(31)-O(31)-H(31O)	106(4)
O(31)-C(31)	1.439(5)	O(31)-C(31)-C(32)	109.0(3)
O(31)-H(31O)	0.98(2)	O(31)-C(31)-C(36)	108.1(3)
C(31)-C(32)	1.514(6)	C(32)-C(31)-C(36)	110.8(3)
C(31)-C(36)	1.516(6)	O(31)-C(31)-H(31A)	109.6000
C(31)-H(31A)	1.0000	C(32)-C(31)-H(31A)	109.6000
O(32)-C(32)	1.434(5)	C(36)-C(31)-H(31A)	109.6000
O(32)-C(37)	1.439(5)	C(32)-O(32)-C(37)	116.1(3)
C(32)-C(33)	1.527(5)	O(32)-C(32)-C(31)	112.2(3)
C(32)-H(32A)	1.0000	O(32)-C(32)-C(33)	105.6(3)
O(33)-C(33)	1.431(4)	C(31)-C(32)-C(33)	111.1(3)
O(33)-C(44)	1.432(5)	O(32)-C(32)-H(32A)	109.3000
C(33)-C(34)	1.529(6)	C(31)-C(32)-H(32A)	109.3000
C(33)-H(33A)	1.0000	C(33)-C(32)-H(32A)	109.3000
O(34)-C(34)	1.430(4)	C(33)-O(33)-C(44)	114.3(3)
O(34)-H(34O)	0.962(19)	O(33)-C(33)-C(32)	112.9(3)
C(34)-C(35)	1.525(5)	O(33)-C(33)-C(34)	106.3(3)
C(34)-H(34A)	1.0000	C(32)-C(33)-C(34)	110.3(3)
O(35)-C(35)	1.436(5)	O(33)-C(33)-H(33A)	109.1000
O(35)-H(35O)	0.98(2)	C(32)-C(33)-H(33A)	109.1000
C(35)-C(36)	1.522(5)	C(34)-C(33)-H(33A)	109.1000
C(35)-H(35A)	1.0000	C(34)-O(34)-H(34O)	112(2)
O(36)-C(36)	1.438(4)	O(34)-C(34)-C(35)	109.4(3)
O(36)-C(52)	1.454(5)	O(34)-C(34)-C(33)	110.2(3)
C(36)-H(36A)	1.0000	C(35)-C(34)-C(33)	111.6(3)
C(37)-C(38)	1.506(6)	O(34)-C(34)-H(34A)	108.5000
C(37)-H(37A)	0.9900	C(35)-C(34)-H(34A)	108.5000
C(37)-H(37B)	0.9900	C(33)-C(34)-H(34A)	108.5000
C(38)-C(43)	1.380(6)	C(35)-O(35)-H(35O)	110(4)
C(38)-C(39)	1.395(6)	O(35)-C(35)-C(36)	109.8(3)
C(39)-C(40)	1.383(6)	O(35)-C(35)-C(34)	107.9(3)
C(39)-H(39A)	0.9500	C(36)-C(35)-C(34)	112.1(3)
C(40)-C(41)	1.388(7)	O(35)-C(35)-H(35A)	109.0000
C(40)-H(40A)	0.9500	C(36)-C(35)-H(35A)	109.0000
C(41)-C(42)	1.378(7)	C(34)-C(35)-H(35A)	109.0000
C(41)-H(41A)	0.9500	C(36)-O(36)-C(52)	116.1(3)

C(42)-C(43)	1.395(6)
C(42)-H(42A)	0.9500
C(43)-H(43A)	0.9500
C(44)-C(45)	1.514(6)
C(44)-H(44A)	0.9900
C(44)-H(44B)	0.9900
C(45)-C(50)	1.369(6)
C(45)-C(46)	1.390(6)
C(46)-C(47)	1.394(6)
C(46)-H(46A)	0.9500
C(47)-C(48)	1.388(6)
C(47)-H(47A)	0.9500
C(48)-C(49)	1.387(6)
C(49)-C(50)	1.389(6)
C(49)-H(49A)	0.9500
C(50)-H(50A)	0.9500
C(51)-H(51A)	0.9800
C(51)-H(51B)	0.9800
C(51)-H(51C)	0.9800
C(52)-C(53)	1.508(6)
C(52)-H(52A)	0.9900
C(52)-H(52B)	0.9900
C(53)-C(54)	1.398(6)
C(54)-C(55)	1.382(7)
C(54)-H(54A)	0.9500
C(55)-C(56)	1.367(8)
C(55)-H(55A)	0.9500
C(56)-C(57)	1.388(8)
C(56)-H(56A)	0.9500
C(57)-H(57A)	0.9500
C(1)-O(1)-H(1O)	105(3)
O(1)-C(1)-C(6)	111.0(3)
O(1)-C(1)-C(2)	109.9(3)
C(6)-C(1)-C(2)	109.5(3)
O(1)-C(1)-H(1A)	108.8000
C(6)-C(1)-H(1A)	108.8000
C(2)-C(1)-H(1A)	108.8000
C(2)-O(2)-C(7)	117.0(3)
O(2)-C(2)-C(1)	112.7(3)
O(2)-C(2)-C(3)	103.7(3)
C(1)-C(2)-C(3)	109.1(3)
O(2)-C(2)-H(2A)	110.4000
C(1)-C(2)-H(2A)	110.4000
C(3)-C(2)-H(2A)	110.4000
C(3)-O(3)-C(14)	113.1(3)
O(3)-C(3)-C(4)	107.5(3)
O(3)-C(3)-C(2)	112.2(3)
C(4)-C(3)-C(2)	108.5(3)
O(3)-C(3)-H(3A)	109.5000
C(4)-C(3)-H(3A)	109.5000
C(2)-C(3)-H(3A)	109.5000
C(4)-O(4)-H(4O)	127(6)
O(4)-C(4)-C(3)	110.2(3)
O(4)-C(4)-C(5)	109.0(3)
C(3)-C(4)-C(5)	112.4(3)

O(36)-C(36)-C(31)	108.4(3)
O(36)-C(36)-C(35)	110.0(3)
C(31)-C(36)-C(35)	110.8(3)
O(36)-C(36)-H(36A)	109.2000
C(31)-C(36)-H(36A)	109.2000
C(35)-C(36)-H(36A)	109.2000
O(32)-C(37)-C(38)	108.9(3)
O(32)-C(37)-H(37A)	109.9000
C(38)-C(37)-H(37A)	109.9000
O(32)-C(37)-H(37B)	109.9000
C(38)-C(37)-H(37B)	109.9000
H(37A)-C(37)-H(37B)	108.3000
C(43)-C(38)-C(39)	118.9(4)
C(43)-C(38)-C(37)	121.2(4)
C(39)-C(38)-C(37)	119.9(4)
C(40)-C(39)-C(38)	121.4(4)
C(40)-C(39)-H(39A)	119.3000
C(38)-C(39)-H(39A)	119.3000
C(39)-C(40)-C(41)	119.7(4)
C(39)-C(40)-H(40A)	120.1000
C(41)-C(40)-H(40A)	120.1000
C(42)-C(41)-C(40)	118.8(4)
C(42)-C(41)-H(41A)	120.6000
C(40)-C(41)-H(41A)	120.6000
C(41)-C(42)-C(43)	121.8(4)
C(41)-C(42)-H(42A)	119.1000
C(43)-C(42)-H(42A)	119.1000
C(38)-C(43)-C(42)	119.4(4)
C(38)-C(43)-H(43A)	120.3000
C(42)-C(43)-H(43A)	120.3000
O(33)-C(44)-C(45)	109.1(3)
O(33)-C(44)-H(44A)	109.9000
C(45)-C(44)-H(44A)	109.9000
O(33)-C(44)-H(44B)	109.9000
C(45)-C(44)-H(44B)	109.9000
H(44A)-C(44)-H(44B)	108.3000
C(50)-C(45)-C(46)	118.7(4)
C(50)-C(45)-C(44)	121.1(4)
C(46)-C(45)-C(44)	120.2(4)
C(45)-C(46)-C(47)	121.0(4)
C(45)-C(46)-H(46A)	119.5000
C(47)-C(46)-H(46A)	119.5000
C(48)-C(47)-C(46)	119.5(4)
C(48)-C(47)-H(47A)	120.3000
C(46)-C(47)-H(47A)	120.3000
O(15)-C(48)-C(49)	115.4(4)
O(15)-C(48)-C(47)	125.1(4)
C(49)-C(48)-C(47)	119.5(4)
C(48)-C(49)-C(50)	120.1(4)
C(48)-C(49)-H(49A)	119.9000
C(50)-C(49)-H(49A)	119.9000
C(45)-C(50)-C(49)	121.1(4)
C(45)-C(50)-H(50A)	119.4000
C(49)-C(50)-H(50A)	119.4000
O(15)-C(51)-H(51A)	109.5000
O(15)-C(51)-H(51B)	109.5000

O(4)-C(4)-H(4A)	108.4000	H(51A)-C(51)-H(51B)	109.5000
C(3)-C(4)-H(4A)	108.4000	O(15)-C(51)-H(51C)	109.5000
C(5)-C(4)-H(4A)	108.4000	H(51A)-C(51)-H(51C)	109.5000
C(5)-O(5)-H(5O)	103(3)	H(51B)-C(51)-H(51C)	109.5000
O(5)-C(5)-C(4)	107.9(3)	O(36)-C(52)-C(53)	112.2(3)
O(5)-C(5)-C(6)	108.8(3)	O(36)-C(52)-H(52A)	109.2000
C(4)-C(5)-C(6)	112.9(3)	C(53)-C(52)-H(52A)	109.2000
O(5)-C(5)-H(5A)	109.1000	O(36)-C(52)-H(52B)	109.2000
C(4)-C(5)-H(5A)	109.1000	C(53)-C(52)-H(52B)	109.2000
C(6)-C(5)-H(5A)	109.1000	H(52A)-C(52)-H(52B)	107.9000
C(6)-O(6)-C(22)	116.2(3)	C(54)-C(53)-C(30)	118.6(4)
O(6)-C(6)-C(1)	108.2(3)	C(54)-C(53)-C(52)	121.0(4)
O(6)-C(6)-C(5)	109.2(3)	C(30)-C(53)-C(52)	120.2(4)
C(1)-C(6)-C(5)	111.9(3)	C(55)-C(54)-C(53)	120.7(5)
O(6)-C(6)-H(6A)	109.1000	C(55)-C(54)-H(54A)	119.7000
C(1)-C(6)-H(6A)	109.1000	C(53)-C(54)-H(54A)	119.7000
C(5)-C(6)-H(6A)	109.1000	C(56)-C(55)-C(54)	120.2(5)
O(2)-C(7)-C(8)	112.2(3)	C(56)-C(55)-H(55A)	119.9000
O(2)-C(7)-H(7A)	109.2000	C(54)-C(55)-H(55A)	119.9000
C(8)-C(7)-H(7A)	109.2000	C(55)-C(56)-C(57)	120.4(5)
O(2)-C(7)-H(7B)	109.2000	C(55)-C(56)-H(56A)	119.8000
C(8)-C(7)-H(7B)	109.2000	C(57)-C(56)-H(56A)	119.8000
H(7A)-C(7)-H(7B)	107.9000	C(56)-C(57)-C(30)	120.1(5)
C(9)-C(8)-C(13)	118.0(4)	C(56)-C(57)-H(57A)	119.9000
C(9)-C(8)-C(7)	121.1(4)	C(30)-C(57)-H(57A)	119.9000
C(13)-C(8)-C(7)	120.9(4)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (≈^{2}x 10^3) for tesc2. The anisotropic displacement factor exponent takes the form: $-2p^2[h^2 a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	30(1)	43(2)	48(2)	-1(1)	-2(1)	-1(1)
C(1)	28(2)	37(2)	42(2)	2(2)	-1(2)	2(2)
O(2)	34(1)	36(2)	38(1)	-1(1)	4(1)	-1(1)
C(2)	30(2)	28(2)	41(2)	2(2)	8(2)	-4(2)
O(3)	28(1)	30(1)	49(2)	1(1)	6(1)	2(1)
C(3)	29(2)	30(2)	42(2)	1(2)	2(2)	1(2)
O(4)	31(1)	34(2)	41(1)	-2(1)	-5(1)	-3(1)
C(4)	30(2)	34(2)	37(2)	-2(2)	-1(2)	1(2)
O(5)	40(2)	28(2)	43(2)	1(1)	5(1)	-2(1)
C(5)	26(2)	35(2)	41(2)	-1(2)	5(2)	-1(2)
O(6)	28(1)	38(2)	47(2)	7(1)	3(1)	5(1)
C(6)	24(2)	30(2)	41(2)	-1(2)	4(2)	8(2)
C(7)	44(2)	38(2)	36(2)	-2(2)	2(2)	-3(2)
C(8)	40(2)	41(2)	39(2)	-5(2)	2(2)	0(2)
C(9)	38(2)	54(3)	65(3)	-14(2)	-1(2)	4(2)
C(10)	51(3)	55(3)	72(3)	-16(3)	7(2)	14(2)
C(11)	64(3)	37(3)	61(3)	-8(2)	4(2)	2(2)
C(12)	58(3)	48(3)	81(4)	-18(3)	8(3)	-8(2)
C(13)	42(2)	47(3)	65(3)	-12(2)	7(2)	-2(2)
C(14)	34(2)	34(2)	63(3)	6(2)	7(2)	1(2)
C(15)	35(2)	32(2)	47(2)	6(2)	3(2)	3(2)
C(16)	33(2)	35(2)	55(2)	-2(2)	11(2)	5(2)
C(17)	33(2)	36(2)	46(2)	1(2)	2(2)	-4(2)
O(18)	35(2)	34(2)	66(2)	-1(1)	-4(1)	5(1)
C(18)	28(2)	32(2)	52(2)	4(2)	0(2)	4(2)
C(19)	39(2)	41(2)	49(2)	-9(2)	1(2)	1(2)
C(20)	35(2)	47(3)	47(2)	1(2)	-2(2)	4(2)
C(21)	42(2)	49(3)	102(4)	-23(3)	2(3)	9(2)
C(22)	36(2)	34(2)	49(2)	8(2)	3(2)	6(2)
C(23)	40(2)	33(2)	43(2)	4(2)	2(2)	2(2)
C(24)	39(2)	41(2)	57(3)	5(2)	5(2)	3(2)
C(25)	43(2)	55(3)	47(2)	4(2)	11(2)	4(2)
C(26)	63(3)	45(3)	51(3)	2(2)	-2(2)	9(2)
C(27)	48(3)	54(3)	54(3)	13(2)	-10(2)	-11(2)
C(28)	38(2)	48(3)	50(2)	13(2)	3(2)	0(2)
O(15)	36(2)	47(2)	57(2)	4(1)	1(1)	-2(1)
C(30)	52(3)	54(3)	49(3)	-9(2)	-2(2)	6(2)
O(31)	28(1)	46(2)	48(2)	2(1)	2(1)	1(1)
C(31)	34(2)	40(2)	44(2)	0(2)	2(2)	3(2)
O(32)	36(2)	41(2)	48(2)	12(1)	5(1)	3(1)
C(32)	32(2)	37(2)	41(2)	1(2)	6(2)	3(2)
O(33)	33(1)	30(2)	56(2)	0(1)	5(1)	-4(1)
C(33)	30(2)	36(2)	41(2)	2(2)	5(2)	-4(2)
O(34)	27(1)	38(2)	46(2)	4(1)	3(1)	0(1)
C(34)	27(2)	38(2)	42(2)	3(2)	2(2)	1(2)
O(35)	37(2)	30(2)	50(2)	-1(1)	4(1)	2(1)
C(35)	28(2)	33(2)	41(2)	0(2)	2(2)	-3(2)
O(36)	33(1)	39(2)	43(2)	0(1)	3(1)	-6(1)
C(36)	28(2)	35(2)	39(2)	4(2)	2(2)	-3(2)
C(37)	37(2)	50(3)	53(3)	11(2)	2(2)	4(2)
C(38)	38(2)	39(2)	37(2)	6(2)	2(2)	3(2)
C(39)	45(2)	45(3)	52(2)	4(2)	5(2)	6(2)

C(40)	61(3)	47(3)	62(3)	19(2)	12(2)	6(2)
C(41)	50(3)	63(3)	50(3)	17(2)	8(2)	-3(2)
C(42)	40(2)	70(3)	46(2)	14(2)	6(2)	5(2)
C(43)	38(2)	43(3)	49(2)	6(2)	4(2)	2(2)
C(44)	35(2)	35(2)	75(3)	-9(2)	7(2)	-3(2)
C(45)	34(2)	31(2)	61(3)	-3(2)	3(2)	-5(2)
C(46)	37(2)	34(2)	58(3)	-2(2)	-2(2)	4(2)
C(47)	39(2)	46(3)	50(2)	1(2)	1(2)	1(2)
C(48)	34(2)	36(2)	48(2)	0(2)	5(2)	0(2)
C(49)	40(2)	50(3)	42(2)	0(2)	1(2)	0(2)
C(50)	41(2)	43(3)	48(2)	8(2)	10(2)	0(2)
C(51)	47(3)	47(3)	75(3)	12(2)	5(2)	-12(2)
C(52)	47(2)	34(2)	46(2)	-2(2)	-2(2)	-6(2)
C(53)	48(2)	35(2)	48(2)	-1(2)	-4(2)	-5(2)
C(54)	46(3)	62(3)	48(3)	-7(2)	-1(2)	-8(2)
C(55)	64(3)	91(4)	47(3)	4(3)	-1(2)	-27(3)
C(56)	95(4)	64(4)	51(3)	9(3)	-9(3)	-27(3)
C(57)	92(4)	44(3)	60(3)	-3(2)	-23(3)	9(3)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (≈^{2}x 10 ^{3}) for tesc2.

	x	y	z	U(eq)
H(1O)	6070(50)	4380(20)	4090(20)	55(14)
H(1A)	3743	4248	3243	43
H(2A)	1506	4942	3877	39
H(3A)	271	4398	2690	40
H(4O)	-3690(40)	3420(40)	2700(60)	180(40)
H(4A)	-1415	3603	3821	40
H(5O)	1090(80)	2460(20)	3060(20)	79(17)
H(5A)	1664	3287	2737	41
H(6A)	2514	3419	4429	38
H(7A)	2635	4492	5391	47
H(7B)	488	4388	5793	47
H(9A)	-2263	5230	5487	63
H(10A)	-2715	6246	5605	71
H(11A)	89	6885	5582	65
H(12A)	3375	6497	5414	74
H(13A)	3835	5477	5299	61
H(14A)	-1294	5313	2597	52
H(14B)	-1243	5457	3532	52
H(16A)	-5227	5044	2037	49
H(17A)	-8334	5547	1824	46
H(19A)	-6833	6624	3692	52
H(20A)	-3737	6116	3909	52
H(21A)	-10300	6761	3608	97
H(21B)	-11578	7062	2873	97
H(21C)	-9182	7209	3030	97
H(22A)	6492	2484	4009	47
H(22B)	4107	2403	4170	47
H(24A)	2900	2635	5472	55
H(25A)	3585	2928	6792	58
H(26A)	6782	3335	7186	64
H(27A)	9360	3380	6284	63
H(28A)	8692	3090	4967	54
H(30A)	8573	7092	9866	62
H(31O)	6820(80)	5940(30)	8570(40)	130(30)
H(31A)	4521	6259	7805	47
H(32A)	2698	5419	8236	44
H(33A)	815	6024	7302	43
H(34O)	-3020(40)	7006(16)	7830(20)	33(10)
H(34A)	-751	6641	8645	43
H(35O)	1410(90)	7970(20)	8060(30)	100(20)
H(35A)	1980	7137	7529	40
H(36A)	3058	6867	9174	41
H(37A)	3479	5613	10124	56
H(37B)	3481	5119	9435	56
H(39A)	2947	4328	10424	57
H(40A)	652	3774	11135	68
H(41A)	-2665	4145	11322	65
H(42A)	-3629	5052	10758	62
H(43A)	-1341	5610	10035	52
H(44A)	26	4882	8194	58
H(44B)	-465	4986	7258	58
H(46A)	-2321	4326	8923	52
H(47A)	-5382	3787	9003	54

H(49A)	-7359	4440	6850	53
H(50A)	-4320	4984	6795	52
H(51A)	-7726	3059	8593	84
H(51B)	-10157	3152	8442	84
H(51C)	-8864	3586	9034	84
H(52A)	4331	7995	9107	51
H(52B)	6766	7930	9035	51
H(54A)	2870	7700	10318	62
H(55A)	3039	7195	11527	81
H(56A)	5914	6636	11904	85
H(57A)	8697	6586	11084	79

Table 6. Torsion angles [∞] for tesc2.

C(7)-O(2)-C(2)-C(1)	76.8(4)	C(37)-O(32)-C(32)-C(31)	-84.2(4)
C(7)-O(2)-C(2)-C(3)	-165.4(3)	C(37)-O(32)-C(32)-C(33)	154.7(3)
O(1)-C(1)-C(2)-O(2)	-70.3(4)	O(31)-C(31)-C(32)-O(32)	59.4(4)
C(6)-C(1)-C(2)-O(2)	51.8(4)	C(36)-C(31)-C(32)-O(32)	-59.4(4)
O(1)-C(1)-C(2)-C(3)	175.0(3)	O(31)-C(31)-C(32)-C(33)	177.3(3)
C(6)-C(1)-C(2)-C(3)	-62.8(4)	C(36)-C(31)-C(32)-C(33)	58.5(4)
C(14)-O(3)-C(3)-C(4)	-172.6(3)	C(44)-O(33)-C(33)-C(32)	-55.4(4)
C(14)-O(3)-C(3)-C(2)	68.1(4)	C(44)-O(33)-C(33)-C(34)	-176.4(3)
O(2)-C(2)-C(3)-O(3)	61.4(4)	O(32)-C(32)-C(33)-O(33)	-53.9(4)
C(1)-C(2)-C(3)-O(3)	-178.2(3)	C(31)-C(32)-C(33)-O(33)	-175.7(3)
O(2)-C(2)-C(3)-C(4)	-57.2(4)	O(32)-C(32)-C(33)-C(34)	64.9(4)
C(1)-C(2)-C(3)-C(4)	63.1(4)	C(31)-C(32)-C(33)-C(34)	-57.0(4)
O(3)-C(3)-C(4)-O(4)	59.8(4)	O(33)-C(33)-C(34)-O(34)	-61.2(4)
C(2)-C(3)-C(4)-O(4)	-178.6(3)	C(32)-C(33)-C(34)-O(34)	176.0(3)
O(3)-C(3)-C(4)-C(5)	-178.3(3)	O(33)-C(33)-C(34)-C(35)	177.0(3)
C(2)-C(3)-C(4)-C(5)	-56.8(4)	C(32)-C(33)-C(34)-C(35)	54.3(4)
O(4)-C(4)-C(5)-O(5)	-66.9(4)	O(34)-C(34)-C(35)-O(35)	63.2(4)
C(3)-C(4)-C(5)-O(5)	170.6(3)	C(33)-C(34)-C(35)-O(35)	-174.5(3)
O(4)-C(4)-C(5)-C(6)	172.9(3)	O(34)-C(34)-C(35)-C(36)	-175.8(3)
C(3)-C(4)-C(5)-C(6)	50.3(4)	C(33)-C(34)-C(35)-C(36)	-53.5(4)
C(22)-O(6)-C(6)-C(1)	141.0(3)	C(52)-O(36)-C(36)-C(31)	-140.3(3)
C(22)-O(6)-C(6)-C(5)	-97.0(4)	C(52)-O(36)-C(36)-C(35)	98.4(4)
O(1)-C(1)-C(6)-O(6)	-62.7(4)	O(31)-C(31)-C(36)-O(36)	63.2(4)
C(2)-C(1)-C(6)-O(6)	175.8(3)	C(32)-C(31)-C(36)-O(36)	-177.4(3)
O(1)-C(1)-C(6)-C(5)	176.9(3)	O(31)-C(31)-C(36)-C(35)	-176.0(3)
C(2)-C(1)-C(6)-C(5)	55.4(4)	C(32)-C(31)-C(36)-C(35)	-56.5(4)
O(5)-C(5)-C(6)-O(6)	71.2(4)	O(35)-C(35)-C(36)-O(36)	-65.9(4)
C(4)-C(5)-C(6)-O(6)	-169.0(3)	C(34)-C(35)-C(36)-O(36)	174.1(3)
O(5)-C(5)-C(6)-C(1)	-168.9(3)	O(35)-C(35)-C(36)-C(31)	174.2(3)
C(4)-C(5)-C(6)-C(1)	-49.2(4)	C(34)-C(35)-C(36)-C(31)	54.3(4)
C(2)-O(2)-C(7)-C(8)	68.0(4)	C(32)-O(32)-C(37)-C(38)	-134.5(4)
O(2)-C(7)-C(8)-C(9)	69.7(5)	O(32)-C(37)-C(38)-C(43)	-25.0(6)
O(2)-C(7)-C(8)-C(13)	-110.7(4)	O(32)-C(37)-C(38)-C(39)	157.0(4)
C(13)-C(8)-C(9)-C(10)	-1.3(7)	C(43)-C(38)-C(39)-C(40)	-0.6(7)
C(7)-C(8)-C(9)-C(10)	178.3(4)	C(37)-C(38)-C(39)-C(40)	177.5(4)
C(8)-C(9)-C(10)-C(11)	0.4(8)	C(38)-C(39)-C(40)-C(41)	-0.3(7)
C(9)-C(10)-C(11)-C(12)	0.7(8)	C(39)-C(40)-C(41)-C(42)	0.9(8)
C(10)-C(11)-C(12)-C(13)	-0.8(8)	C(40)-C(41)-C(42)-C(43)	-0.7(8)
C(11)-C(12)-C(13)-C(8)	-0.2(8)	C(39)-C(38)-C(43)-C(42)	0.8(6)
C(9)-C(8)-C(13)-C(12)	1.2(7)	C(37)-C(38)-C(43)-C(42)	-177.3(4)
C(7)-C(8)-C(13)-C(12)	-178.4(4)	C(41)-C(42)-C(43)-C(38)	-0.2(7)
C(3)-O(3)-C(14)-C(15)	168.9(3)	C(33)-O(33)-C(44)-C(45)	-177.3(3)
O(3)-C(14)-C(15)-C(20)	120.7(4)	O(33)-C(44)-C(45)-C(50)	74.9(5)
O(3)-C(14)-C(15)-C(16)	-62.9(5)	O(33)-C(44)-C(45)-C(46)	-106.1(4)
C(20)-C(15)-C(16)-C(17)	1.9(6)	C(50)-C(45)-C(46)-C(47)	0.7(6)
C(14)-C(15)-C(16)-C(17)	-174.6(4)	C(44)-C(45)-C(46)-C(47)	-178.3(4)
C(15)-C(16)-C(17)-C(18)	-0.5(6)	C(45)-C(46)-C(47)-C(48)	1.0(6)
C(21)-O(18)-C(18)-C(19)	-0.2(6)	C(51)-O(15)-C(48)-C(49)	178.7(4)
C(21)-O(18)-C(18)-C(17)	-179.9(4)	C(51)-O(15)-C(48)-C(47)	-2.0(6)
C(16)-C(17)-C(18)-C(19)	-0.7(6)	C(46)-C(47)-C(48)-O(15)	178.6(4)
C(16)-C(17)-C(18)-O(18)	179.0(3)	C(46)-C(47)-C(48)-C(49)	-2.1(6)
O(18)-C(18)-C(19)-C(20)	-179.2(4)	O(15)-C(48)-C(49)-C(50)	-179.0(4)
C(17)-C(18)-C(19)-C(20)	0.4(6)	C(47)-C(48)-C(49)-C(50)	1.6(6)
C(18)-C(19)-C(20)-C(15)	1.0(7)	C(46)-C(45)-C(50)-C(49)	-1.3(6)
C(16)-C(15)-C(20)-C(19)	-2.1(6)	C(44)-C(45)-C(50)-C(49)	177.7(4)

C(14)-C(15)-C(20)-C(19)	174.4(4)	C(48)-C(49)-C(50)-C(45)	0.2(7)
C(6)-O(6)-C(22)-C(23)	-65.3(4)	C(36)-O(36)-C(52)-C(53)	54.4(5)
O(6)-C(22)-C(23)-C(28)	-80.1(5)	C(57)-C(30)-C(53)-C(54)	1.9(6)
O(6)-C(22)-C(23)-C(24)	97.2(4)	C(57)-C(30)-C(53)-C(52)	-173.5(4)
C(28)-C(23)-C(24)-C(25)	1.1(6)	O(36)-C(52)-C(53)-C(54)	-103.7(5)
C(22)-C(23)-C(24)-C(25)	-176.2(4)	O(36)-C(52)-C(53)-C(30)	71.6(5)
C(23)-C(24)-C(25)-C(26)	0.6(7)	C(30)-C(53)-C(54)-C(55)	-1.8(7)
C(24)-C(25)-C(26)-C(27)	-2.4(7)	C(52)-C(53)-C(54)-C(55)	173.5(4)
C(25)-C(26)-C(27)-C(28)	2.5(7)	C(53)-C(54)-C(55)-C(56)	0.6(8)
C(26)-C(27)-C(28)-C(23)	-0.7(7)	C(54)-C(55)-C(56)-C(57)	0.7(8)
C(24)-C(23)-C(28)-C(27)	-1.1(6)	C(55)-C(56)-C(57)-C(30)	-0.6(8)
C(22)-C(23)-C(28)-C(27)	176.3(4)	C(53)-C(30)-C(57)-C(56)	-0.7(7)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for tesc2 [≈ and ∞].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1O)...O(3)#1	0.961(19)	1.92(3)	2.815(4)	154(4)
O(4)-H(4O)...O(6)#2	0.97(2)	2.08(8)	2.773(4)	127(8)
O(5)-H(5O)...O(34)#3	0.97(2)	1.79(2)	2.742(4)	164(5)
O(31)-H(31O)...O(33)#1	0.98(2)	1.75(2)	2.729(4)	173(7)
O(34)-H(34O)...O(36)#2	0.962(19)	1.80(2)	2.748(4)	169(3)
O(35)-H(35O)...O(4)#4	0.98(2)	1.77(2)	2.737(4)	170(6)

Symmetry transformations used to generate equivalent atoms:
#1 x+1,y,z #2 x-1,y,z #3 -x,y-1/2,-z+1 #4 -x,y+1/2,-z+1